Supporting Information

Imaging cellular distribution of Bcl inhibitors using small molecule drug conjugates

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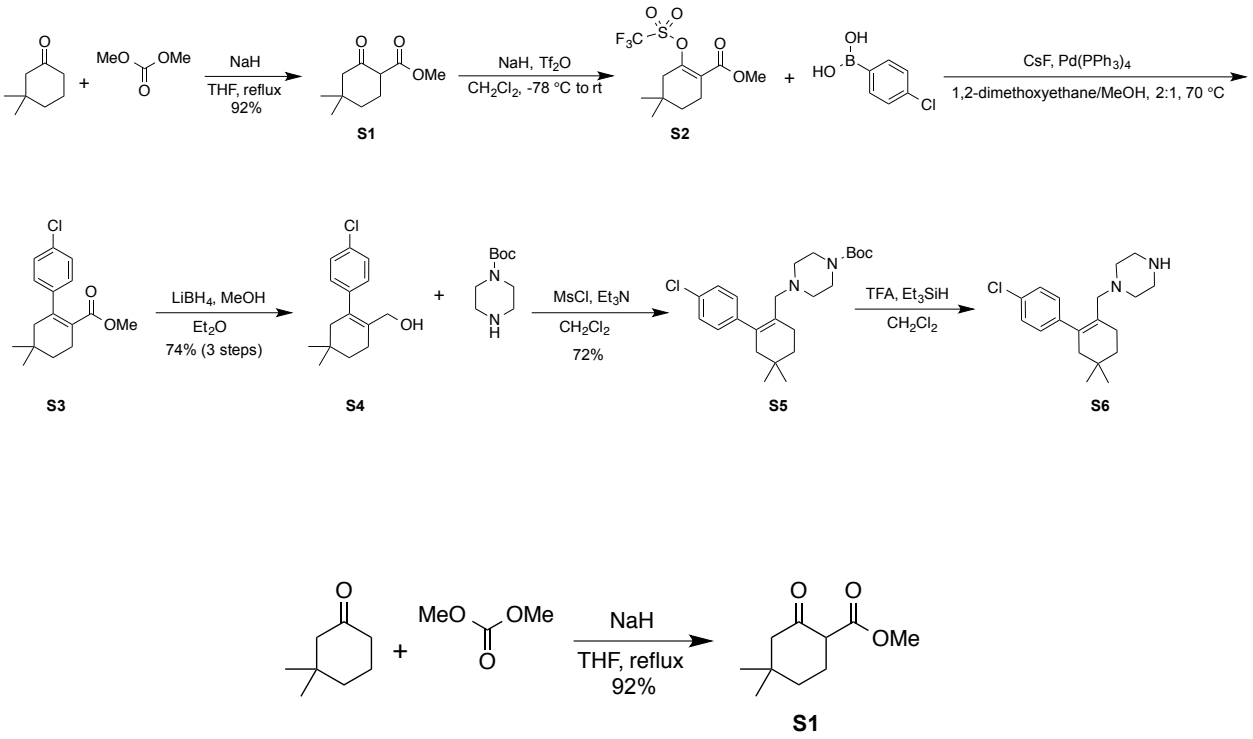
Materials and Instrumentation.

Moisture-sensitive reactions were performed under an atmosphere of nitrogen or argon. Unless otherwise noted, all reaction solvents were purchased as anhydrous grade from Sigma-Aldrich, and starting materials and reagents were purchased from commercial suppliers (primarily Sigma-Aldrich and Life Technologies). Analytical thin-layer chromatography (TLC) was performed on pre-coated silica gel 60 F-254 plates (particle size 0.040–0.050 mm, 230–400 mesh) and visualization was accomplished by staining with $KMnO_4$ or *p*-anisaldehyde solutions or by visualizing under a UV lamp. NMR spectra were recorded on a Bruker Ascend™ 400 MHz instrument. ^{1}H NMR spectra were referenced to residual chloroform (^{1}H, 7.26 ppm; ^{13}C, 77.0 ppm) or DMSO (^{1}H, 2.50; ^{13}C, 39.5) and chemical shifts (δ) are reported in ppm using the following convention: chemical shift, (multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b =broad), coupling constants, and integration). A Biotage® Isolera™ Four

system was used for performing separations by automated chromatography, and reverse-phase chromatography was accomplished using C18 cartridges purchased from Fisher Scientific (KP-C18-HS; 21x 55 mm, 12 g). High performance liquid chromatography-mass spectroscopy data (HPLC-MS) were obtained using a Waters (Milford, MA) instrument equipped with a Waters 2424 ELS Detector, Waters 2998 UV-Vis diode array detector, The synthesis of the acid **1** (the core of ABT-199) was carried out according to reported protocols.[1,2] Detailed procedures are described herein. Waters 2475 multi-wavelength fluorescence detector, and a Waters 3100 mass detector. For analytical separations, a Waters XTerra® C18 5 µm column was used (eluents 0.1% formic acid (v/ v) in water and MeCN; gradient: 0–1.5 min, 5–100% then 1.5–2.0 min 100%.

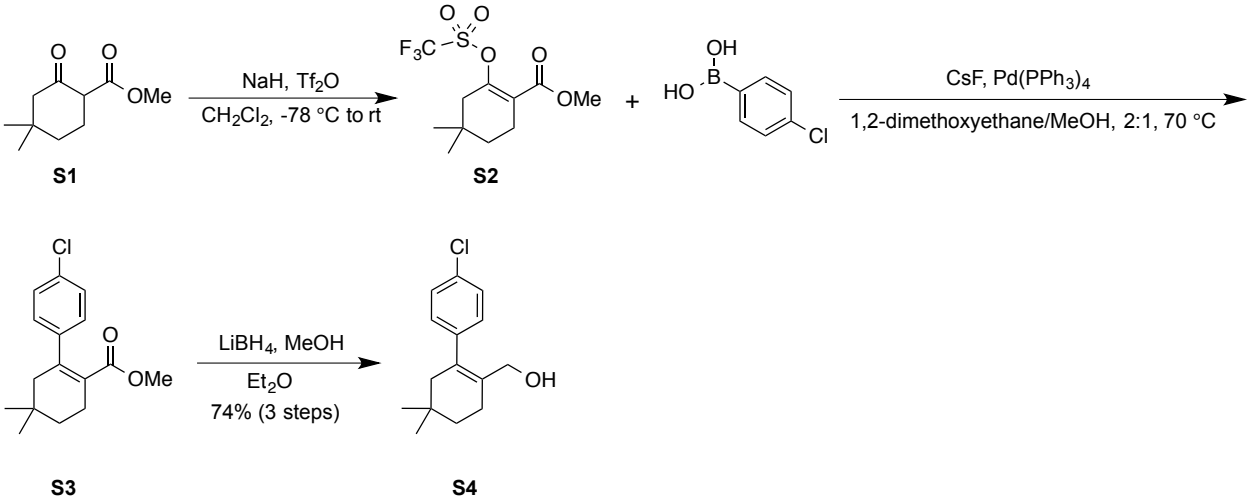
Chemical Synthesis.

Scheme S1. Synthesis of piperazine **S6**.



Methyl 4,4-dimethyl-2-oxocyclohexanecarboxylate (S1). The compound was synthesized using a modified protocol.[3] An oven-dried 250-mL round-bottom 3-necked flask equipped with a condenser and an addition funnel was maintained under an Ar atmosphere and charged with anhydrous THF (72 mL), NaH (1.9 g, 48 mmol, 60% dispersion in mineral oil) and dimethyl carbonate (10 mL, 119 mmol). The suspension was heated to reflux, and a solution of 3,3-dimethylcyclohexanone (3.30 mL, 23.8 mmol) in anhydrous THF (36 mL) was added (slowly,

via the addition funnel). Following the addition, the solution was heated at reflux for 2 h, cooled to room temperature, and poured onto saturated aqueous NH_4Cl (100 mL). The mixture was diluted with Et_2O (200 mL), and the aqueous layer was extracted with Et_2O (2 x 100 mL). The combined organic layers were washed with water and brine, dried ($MgSO_4$), and concentrated *in vacuo*. Purification by chromatography on SiO_2 (hexanes/EtOAc, 95:5) afforded **S1** (4.05 g, 92%) as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.11 (s, 1 H), 3.76 (s, 3 H), 2.24 (t, J = 6.3 Hz, 2 H), 2.05 (s, 2 H), 1.38 (t, J = 6.5 Hz, 2 H), 0.95 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (enol) 173.0, 171.4, 96.1, 51.3, 42.6, 35.1, 29.6, 27.9 (keto) 206.0, 170.4, 56.1, 54.4, 52.1, 36.6, 36.4, 29.9, 27.0, 25.5.



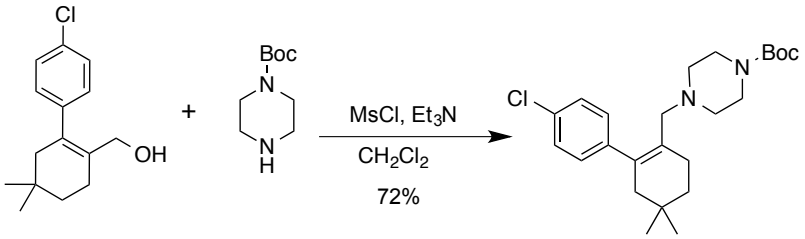
(4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methanol (S4) (Synthesized in 3 steps from S1):

Methyl 4,4-dimethyl-2-(((trifluoromethyl)sulfonyl)oxy)cyclohex-1-enecarboxylate (S2). An oven-dried 50-mL round-bottom flask was charged with CH_2Cl_2 (18 mL) and NaH (436 mg, 10.9 mmol, 60% dispersion in mineral oil) and cooled to 0 ºC. Methyl 4,4-dimethyl-2-oxocyclohexanecarboxylate (**S1**) (1.00 g, 5.43 mmol) was added drop-wise via syringe. The slurry was stirred for 30 min at 0 ºC and then cooled to -78 ºC. Triflic anhydride (1.00 mL, 5.97 mmol) was added slowly, and the reaction mixture was stirred overnight, warming to room temperature as it stirred. The reaction mixture was quenched with brine (slowly—foaming and emulsions were observed!) and transferred to a 500-mL Erlenmeyer flask (using CH_2Cl_2 as transfer solvent) and quenched with additional brine (300 mL of CH_2Cl_2, 150 mL of brine). The layers were separated, and the organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford crude **S2** (1.71 g, quant.) as a pale yellow oil which was carried forward to the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 3 H), 2.53–2.48 (m, 2 H), 2.18 (t, J = 2.4 Hz, 2 H), 1.44 (t, J = 6.4 Hz, 2 H), 1.01 (s, 3 H), 1.01 (s, 3 H).

Methyl 4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-carboxylate (S3). An oven-dried vessel equipped with stir bar was cooled under an Ar atmosphere and charged with a solution of enol triflate **S2** (1.71 g, 5.41 mmol) in DME/MeOH, 2:1 (13.2 mL DME, 6.6 mL MeOH). The solution was sparged with Ar for 30 min, and 4-chlorophenylboronic acid (846 mg, 5.41 mmol), CsF (1.64 g, 10.8 mmol), and $Pd(PPh_3)_4$ (185 mg, 0.160 mmol) were added. The

ºC for 20 h. Upon cooling, the solvent was removed in vacuo, Et_2O (20 mL) was added, and the solids were removed by filtration through Celite. The filtrate was concentrated in vacuo to afford crude **S3** (1.76 g) as a brown oil which was carried forward without further purification.

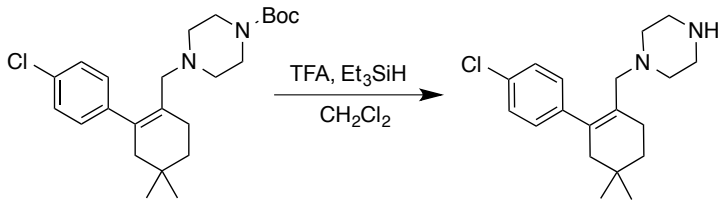
(4'-Chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methanol (Synthesized in 3 steps from S1). A 125-mL wide-mouthed round-bottom flask was charged with a solution of crude **S3 (**1.51 g, 5.41 mmol, assuming quant. yield from the previous step) in Et_2O (10 mL) was treated with $LiBH_4$ (353 mg, 16.2 mmol) followed by drop-wise addition of MeOH (caution—this addition must be done with care to avoid generating a large exotherm/loss of material over the sides of the vessel). The mixture was stirred vigorously at room temperature for 48 h and then quenched (carefully) with 1 M aqueous HCl (~15 mL total) and extracted into Et_2O (4 x 50 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Purification by chromatography on SiO_2 (hexanes/EtOAc, 95:5, then 4:1) afforded **S4** (1.01 g, 74% over 3 steps) as a viscous pale yellow oil that solidifies (white solid) upon standing: R_f 0.32 (hexanes/EtOAc, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.1 Hz, 2 H), 7.06 (d, *J* = 8.4 Hz, 2 H), 3.94 (s, 2 H), 2.31-2.28 (m, 2 H), 2.04 (s, 2 H), 1.49 (t, *J* = 6.5 Hz, 2 H), 1.38 (bs, 1 H), 0.98 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.4, 134.6, 132.3, 131.7, 129.4, 128.2, 63.4, 46.1, 35.2, 29.1, 28.0, 24.8.



S4 **S5**

***tert*-Butyl 4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazine-1-carboxylate (S5).** An oven-dried 50-mL round-bottom flask was charged with a solution of **S4** (661 mg, 2.64 mmol), in anhydrous CH_2Cl_2 (13.7 mL). Et_3N (0.68 mL, 4.9 mmoL) was added, and the solution was cooled to 0 ºC. MsCl (0.23 mL, 3.0 mmol) was added, followed by 1-*N*-Boc-piperazine (610 mg, 3.28 mmol) were added. The solution was stirred overnight (16 h), warming to room temperature as it stirred. After the 16 h, residual mesylate was present (TLC), and additional 1-*N*-Boc-piperazine (305 mg) were added, followed by Et_3N (0.6 mL). Note: The solution (which had been homogeneous) became cloudy upon addition of Et_3N. The mixture was stirred for an additional 24 h at room temperature and was subsequently diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 9:1 to 8:2) to afford **S5** (790 mg, 72%) as a colorless oil: R_f 0.30 (hexanes/EtOAc, 8:2) Note: the product "streaks" under these conditions; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.6 Hz, 2 H), 6.96 (d, *J* = 8.6 Hz, 2 H), 3.36 (app t, *J* = 5.0 Hz, 4 H), 2.78 (s, 2 H), 2.22 (app t, *J* = 6.5 Hz, 2 H), 2.16 (app t, *J* = 5.1 Hz, 4 H). 2.00 (s, 2 H), 1.47-1.42 (m, 11 H), 0.97 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 142.1, 134.8, 131.8, 129.7, 129.3, 128.1, 79.3, 60.4, 52.6, 46.9, 44.0,

43.3, 35.3, 29.1, 28.4, 28.1, 25.5; LC-MS (ESI) *m/z* calc for $C_{24}H_{36}ClN_2O_2$ $[M+H]^+$ 419.25, found 419.33.

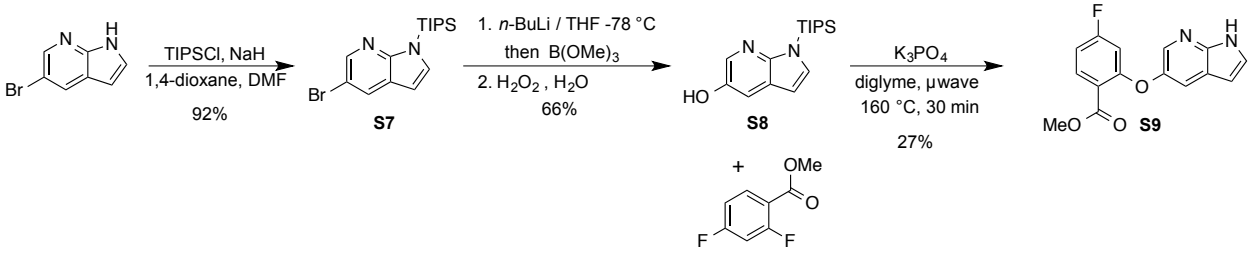


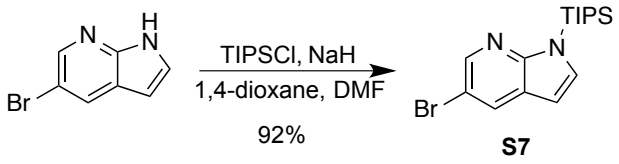
S5 **S6**

1-((4'-Chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazine (S6). A solution of compound **S5** (200 mg, 0.477 mmol) in CH_2Cl_2 (15 mL) was treated with Et_3SiH (1 mL) and TFA (15 mL) and stirred at room temperature for 90 min. The solvents were removed *in vacuo*, and the residue was taken up in EtOAc and washed (2x) with saturated aqueous NaH_2PO_4 and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was coevaporated with toluene (twice), and the crude residue was taken forward to the next step without further purification. LC-MS (ESI) *m/z* calc for $C_{19}H_{28}ClN_2$ $[M+H]^+$ 319.19, found 319.37.

Note: The product likely exists as a salt; subsequent calculations are based on the MW of the free base.

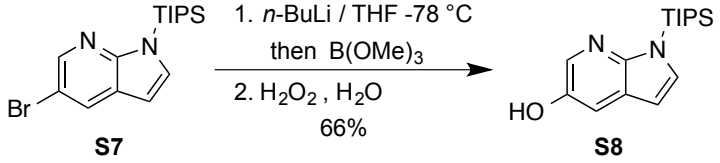
Scheme S2. Synthesis of 7-azaindole fragment **S9**





S7

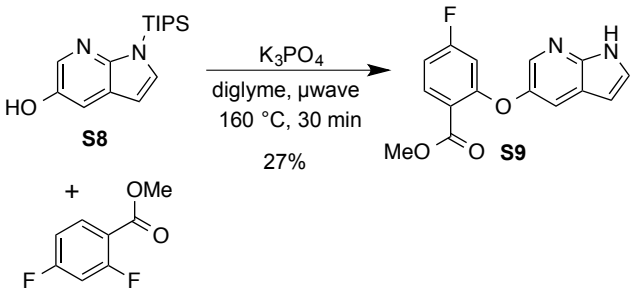
5-Bromo-1-(triisopropylsilyl)-1*H*-pyrrolo[2,3-b]pyridine (S7). An oven-dried 250-mL round-bottom flask was charged with 5-bromo-7-azaindole (2.00 g, 10.2 mmol), 1,4-dioxane (20 mL), and DMF (6.5 mL). The solution was treated (portion-wise) with NaH (490 mg, 12.2 mmol, 60% dispersion in mineral oil), and the resulting yellow slurry was stirred at room temperature for 1 h. TIPSCl (3.5 mL, 16 mmol) was added, and the slurry was stirred at room temperature overnight. The mixture was poured into water (150 mL), the aqueous mixture was extracted with CH_2Cl_2 (3 x 100 mL), and the combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Purification by chromatography on SiO_2 (hexanes/EtOAc, 95:5) afforded **S7** (3.31 g, 92%) as a white solid. Spectral data were in agreement with previously reported data.[4] R_f 0.6 (hexanes/EtOAc, 95:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27-8.26 (m, 1 H), 7.98-7.97 (m, 1 H), 7.30 (d, *J* = 3.5 Hz, 1 H), 6.49 (d, *J* = 3.5 Hz, 1 H), 1.83 (sept, *J* = 7.5 Hz, 3 H), 1.11 (d, *J* = 7.4 Hz, 18 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.1, 142.8, 132.7, 129.8, 124.0, 112.0, 102.5, 18.1, 12.2; LC-MS (ESI) *m/z* calc for $C_{16}H_{26}BrN_2Si$ $[M+H]^+$ 353.10, found 353.12.



S7 **S8**

1-(Triisopropylsilyl)-1*H*-pyrrolo[2,3-*b*]pyridin-5-ol (S8). An oven-dried 250-mL round-bottom flask was charged with **S7** (2.80 g, 7.92 mmol) and anhydrous THF (60 mL). The solution was cooled to -78 ºC and stirred for 30 min. *n*-BuLi (3.5 mL, 8.7 mmil, c = 2.5 M in hexanes) was added slowly via syringe. [Note: the solution became bright yellow in color.] After stirring for 5 min, $B(OMe)_3$ (1.3 mL, 12 mmol) was added drop-wise [Note: the yellow color dissipated after adding *ca.* 0.9 mL $B(OMe)_3$.] The ice-bath was removed, and the solution was stirred for 1 h, warming to room temperature as it stirred. The solution was poured into water (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine and concentrated *in vacuo*. The residue was coevaporated with THF (1 x 10 mL), taken up in THF (24 mL), and cooled to 0 ºC and stirred for 15 min, after which 1 M aqueous NaOH (8 mL) and 30% H_2O_2 (1 mL) were added. The solution was stirred for 1 h at 0 ºC, and then $Na_2S_2O_3$ (1.2 g) was added, followed by concentrated aqueous HCl and solid NaH_2PO_4 to bring the pH to between 4 and 5. The solution was extracted with EtOAc, and the combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by chromatography on SiO_2 (hexanes/EtOAc, 95:5, then 4:1) afforded **S8** (1.52 g, 66%) as a viscous pink oil that solidifies upon standing: R_f 0.38 (hexanes/EtOAc, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 2.7 Hz, 1 H), 7.32 (d, *J* = 2.6 Hz, 1 H), 7.28 (d, *J* = 3.4 Hz, 1 H), 6.44 (d, *J* = 3.2 Hz, 1 H), 4.44 (s, 1 H), 1.82 (sept, *J* = 7.5 Hz, 3 H), 1.11 (d, *J* = 7.5 Hz, 18 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.5, 146.6, 132.5, 131.9, 122.6, 113.2, 102.2, 18.1, 12.3; LC-MS (ESI) *m/z* calc for $C_{16}H_{27}N_2OSi$ $[M+H]^+$ 291.19, found 291.24.

The ^{1}H NMR spectrum of compound **S8** was also measured in DMSO-d_6; the spectral data were in agreement with previously reported data:[5] ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1 H), 7.82-7.81 (m, 1 H), 7.36-7.35 (m, 1 H), 7.27-7.26 (m, 1 H), 6.44-6.43 (m, 1 H), 1.81 (sept, *J* = 7.5 Hz, 3 H), 1.05 (d, *J* = 7.4 Hz, 18 H).



Methyl 2-((1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)oxy)-4-fluorobenzoate (S9). An oven-dried microwave vial was charged with **S8** (500 mg, 1.72 mmol), diglyme (8.0 mL), methyl 2,4-difluorobenzoate (300 μL, 2.41 mmol), and K_3PO_4 (548 mg, 2.58 mmol). The solution was purged with argon for 10 min and then subjected to microwave heating at 160 ºC for 30 min. Upon cooling, the dark brown solution was diluted with water (50 mL) and Et_2O (50 mL) and the aqueous layer was extracted with Et_2O (3 x 30 mL). The combined organic layers were washed with water (3 x), saturated aqueous LiCl, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Purification by chromatography (Biotage, 25 g SiO_2 cartridge, linear gradient of 2% EtOAc/ hexanes to 50% EtOAc/hexanes over 15 min) afforded a residue that was coevaporated with toluene to afford **S9** (135 mg, 27%) as a tan solid: ^{1}H NMR (400 MHz, CDCl3) δ 10.69 (s, 1 H), 8.21 (d, *J* = 2.5 Hz, 1 H), 7.97, 7.96 (AB *J* = 6.8 Hz, 1 H), 7.67 (d, *J* = 2.6 Hz, 1 H), 7.43 (t, *J* = 2.9 Hz, 1 H), 6.83–6.79 (m, 1 H), 6.53–6.49 (m, 2 H) 3.90 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6 (d, *J* = 252.7 Hz), 165.4, 160.3 (d, *J* = 10.3 Hz) 146.8, 146.1, 136.2, 134.0 (d, *J* = 10.5 Hz), 127.0, 120.8, 120.1, 117.6 (d, *J* = 3.2 Hz), 109.6 (d, *J* = 21.8 Hz), 105.4 (d, *J* = 25.4 Hz), 101.0, 52.2; LC-MS (ESI) *m/z* calc for $C_{15}H_{12}FN_2O_3$ $[M+H]^+$ 287.08, found 287.07.

The S_NAR reaction of 2,4-difluorobenzoates and related carbonyl analogs with alkoxide nucleophiles has been shown to occur preferentially at the *ortho*-position.[6] To confirm the regioselectivity, the C-F coupling constants were used in conjugation with COSY and HSQC spectral data. The position of the aromatic protons was determined using COSY and HSCQ; the proton corresponding to Ha was shown to be attached to the carbon with *J* = 25.4 Hz, Hb was attached to the carbon with *J* = 21.8 Hz. Because the coupling constants indicate that these carbons are *ortho* to the carbon bearing the fluorine, it is assumed that *ortho*-selectivity of the SNAR was achieved. Furthermore, the carbons bearing the coupling constants of 21.8 Hz and 25.4 Hz are both attached to protons via a DEPT-135 experiment (attached proton test). The HSQC and ^{13}C data for compound **S9** are also analogous to those obtained for a

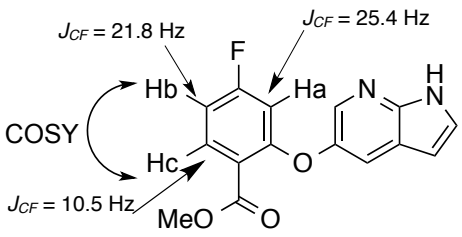


Figure S1. Assignment of regioselectivity of **S9.**

commercial sample of methyl 4-fluorosalicylic acid.

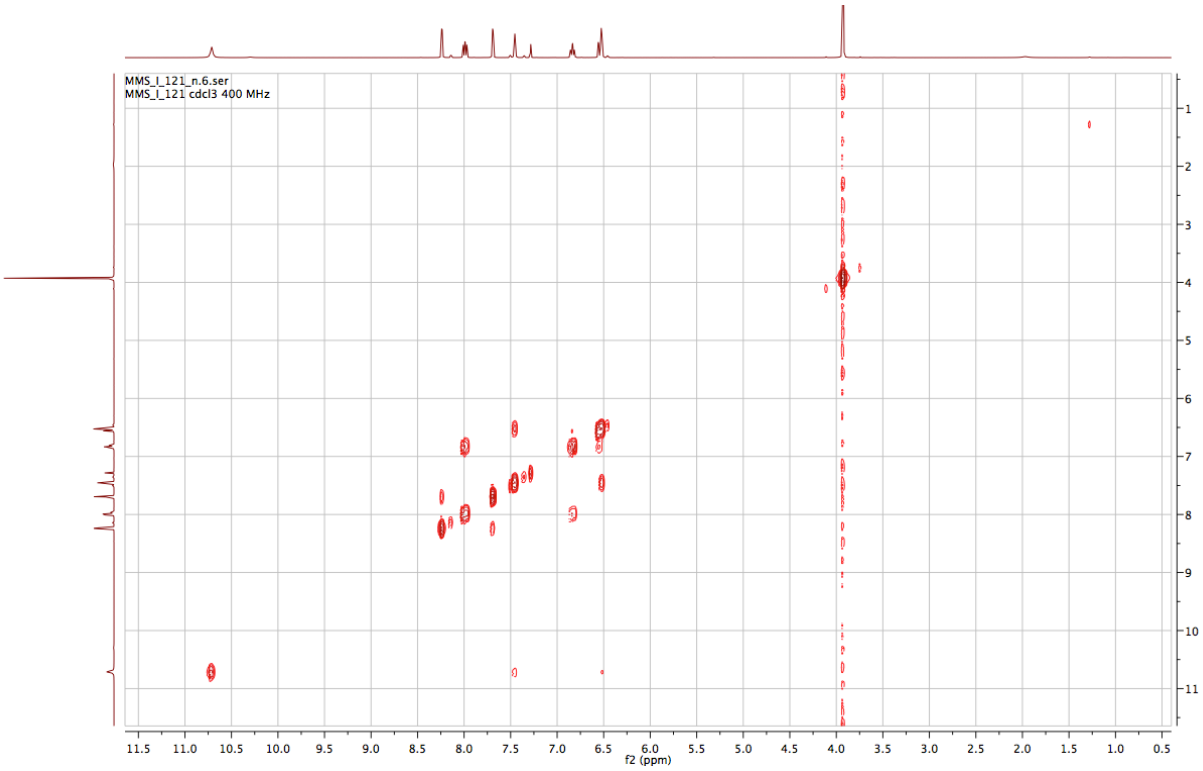


Figure S2. COSY spectral data for compound **S9.**

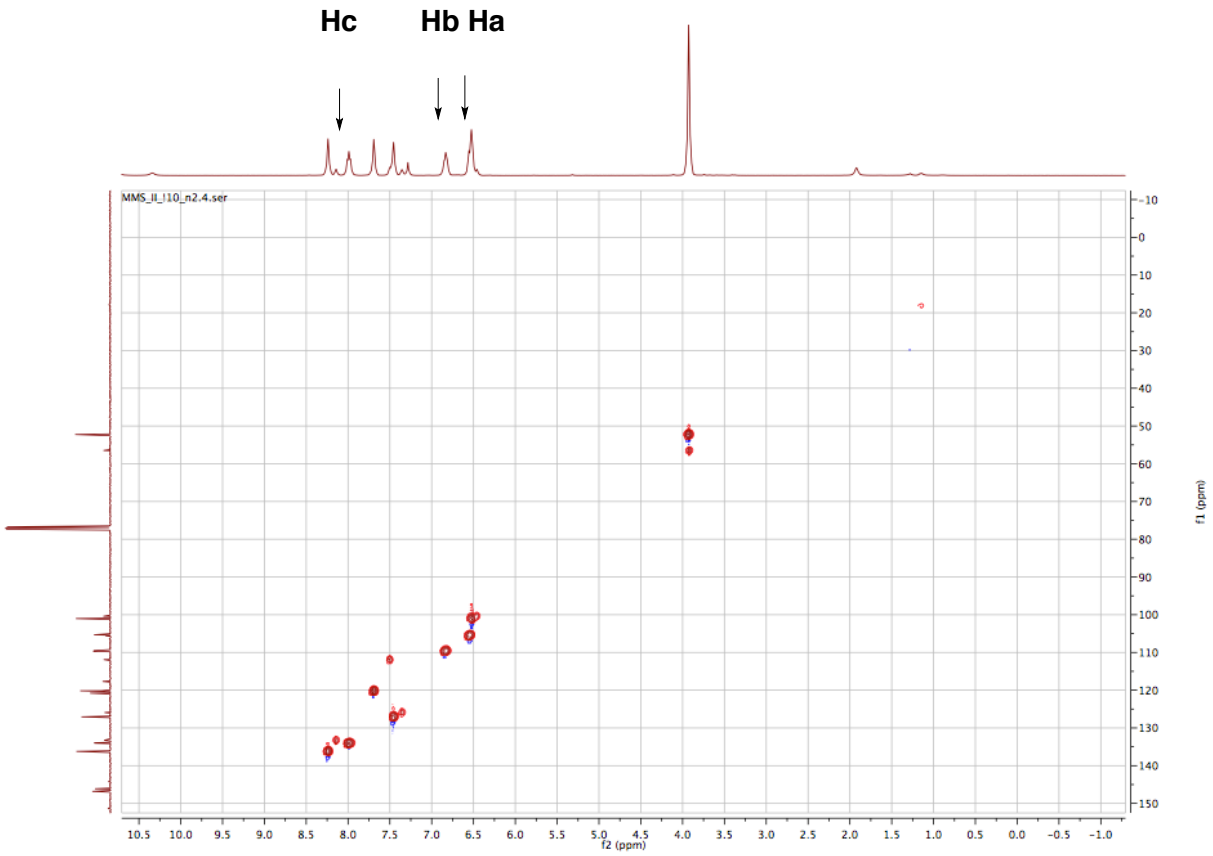
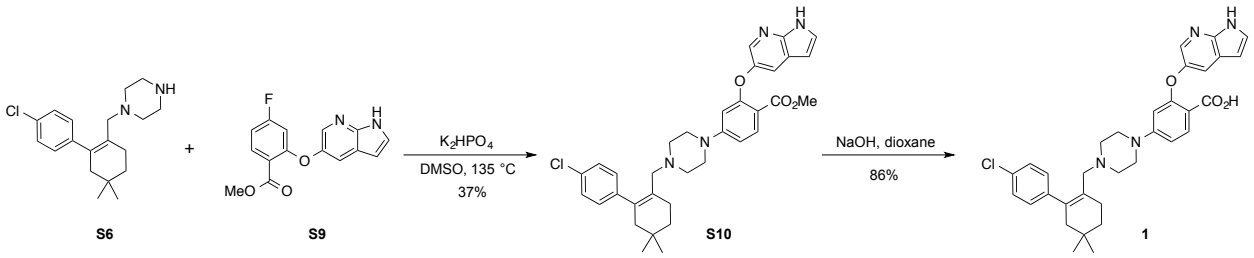
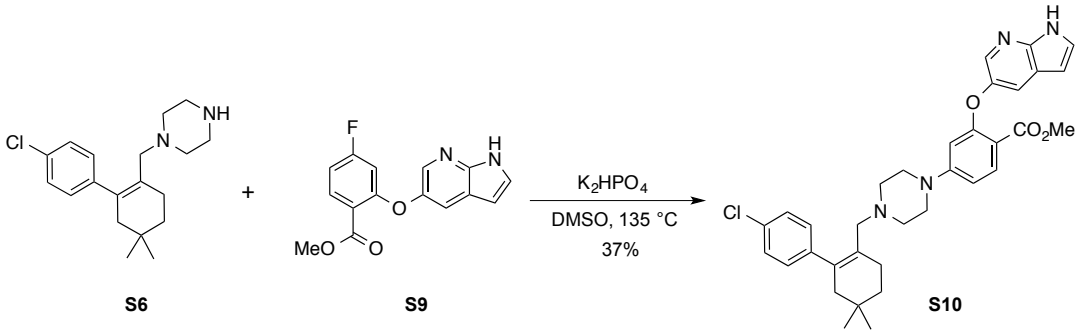


Figure S3. HSQC spectral data for compound **S9**.

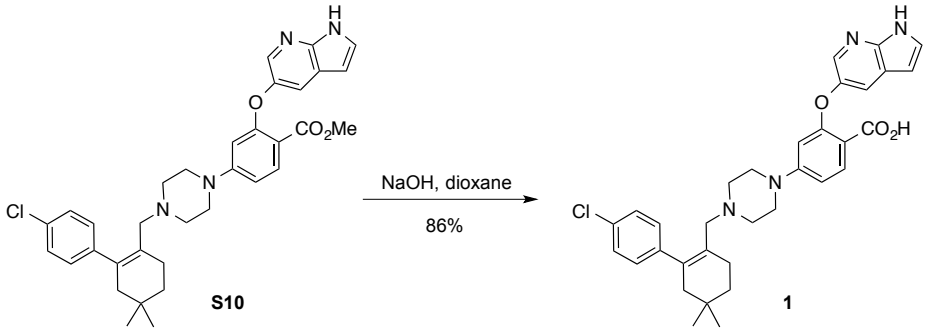
Scheme S3. Synthesis of acid **1** via SNAR reaction of **S6** and **S9** followed by hydrolysis.





S6 **S9** **S10**

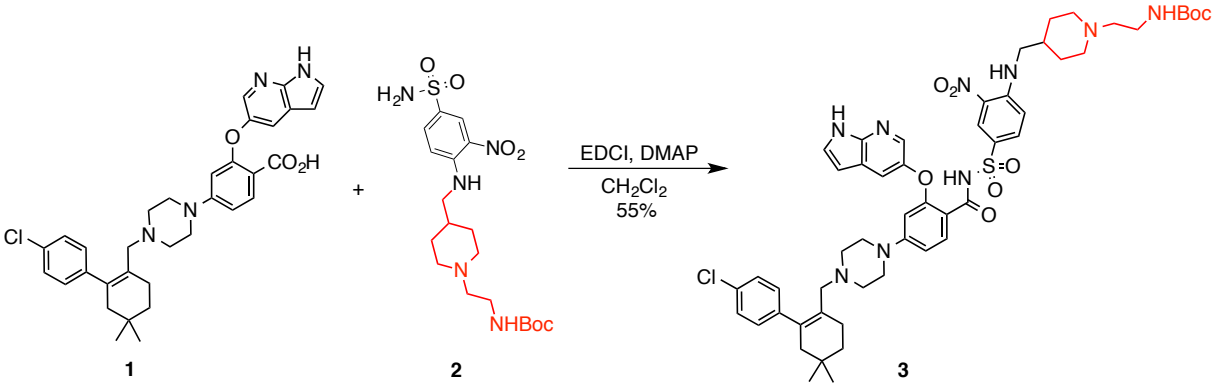
Methyl 2-((1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)oxy)-4-(4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoate (S10). An oven-dried vial was charged with aryl fluoride **S9** (68 mg, 0.24 mmol), amine **S6** (ca. 152 mg, 0.48 mmol, assuming quantitative conversion from deprotection of **S5**, based on the MW of the free base), anhydrous DMSO (1.2 mL), and K_2HPO_4 (104 mg, 0.598 mmol). The solution was purged with Ar for 10 min, sealed, and heated at 135 ºC for 19 h. Upon cooling, the solution was diluted with Et_2O (30 mL) and washed with 1 M aqueous NaOH (3 x 10 mL) and brine (10 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by chromatography (Biotage, 10 g SiO_2 column, hexanes/EtOAc, 9:1 to 4:6) to afford **S10** (51 mg, 37%) as an off-white solid: R_f 0.15 (hexanes/EtOAc, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1 H), 8.16 (s, 1 H), 7.88 (d, *J* = 8.8 Hz, 1 H), 7.50 (s, 1 H), 7.32 (obscured, 2 H), 6.94 (d, *J* = 7.9 Hz, 2 H), 6.58 (d, *J* = 9.1, 2.6 Hz, 1 H), 6.45-6.43 (m, 1 H), 6.27 (s, 1 H), 3.78 (s, 3 H), 3.14-3.11 (m, 4 H), 2.77 (s, 2 H), 2.28-2.25 (m, 4 H), 2.21-2.18 (m, 2 H), 1.98 (bs, 2 H), 1.43 (t, *J* = 6.5 Hz, 2 H), 0.95 (s, 6 H); LC-MS (ESI) *m/z* calc for $C_{34}H_{38}ClN_4O_3$ 585.26 $[M+H]^+$, found 585.38.



S10 **1**

2-((1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)oxy)-4-(4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoic acid 1. A solution of methyl ester **S10** (51.4 mg, 0.0878 mmol) in 1,4-dioxane (2.5 mL) and 1 M aqueous NaOH (1.5 mL) was heated at 50 ºC for 20 h. Upon cooling, the solution was poured into saturated aqueous NaH_2PO_4 (20 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was coevaporated with toluene (2 x) to afford acid **1** (43.1 mg, 86%) as a tan solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ

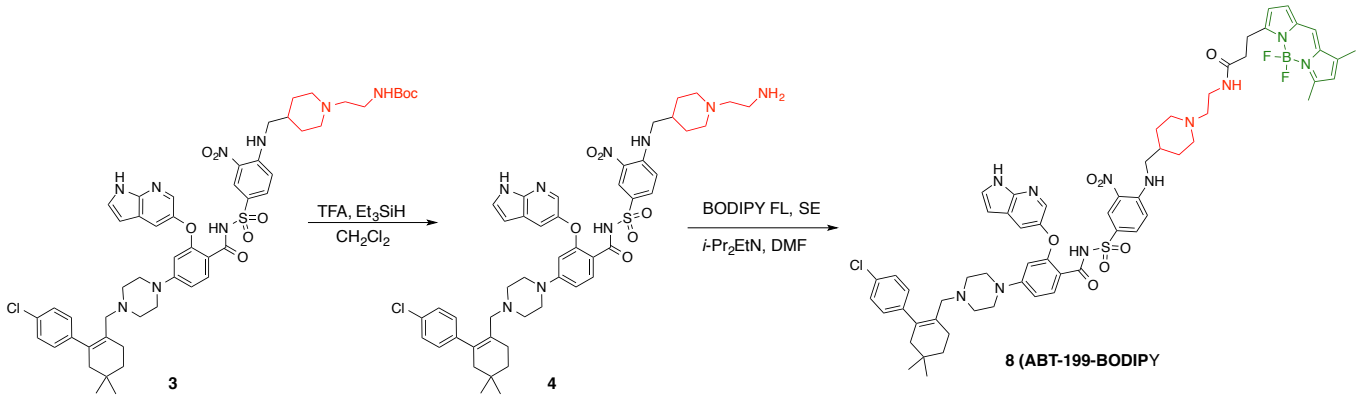
10.24 (bs, 1 H), 8.17 (s, 1 H), 8.00 (d, *J* = 8.9 Hz, 1 H), 7.60 (s, 1 H), 7.34 (s, 1 H), 7.29 (d, *J* = 7.9 Hz, 2 H), 6.93 (d, *J* = 7.9 Hz, 2 H), 6.61 (d, *J* = 8.9 Hz, 1 H), 6.42-6.41 (m, 1 H), 6.21 (s, 1 H), 3.14 (t, *J* = 4.6 Hz, 4 H), 2.80 (s, 2 H), 2.28 (t, *J* = 4.8 Hz, 4 H), 2.19 (t, *J* = 6.6 Hz, 2 H), 1.98 (s, 2 H), 1.42 (t, *J* = 6.4 Hz, 2 H), 0.94 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 159.5, 155.4, 147.1, 145.2, 142.1, 135.4, 134.6, 132.0, 129.7, 129.0, 128.2, 128.2, 127.1, 120.9, 119.7, 109.0, 109.2, 103.2, 100.9, 60.3, 52.3, 47.0, 47.0, 35.3, 29.2, 28.2, 25.6; LC-MS (ESI) *m/z* calc for $C_{33}H_{36}ClN_4O_3$ $[M+H]^+$ 571.25, found 571.38.



***tert*-Butyl (2-(4-(((4-(N-(2-((1*H*-pyrrolo[2,3-b]pyridin-5-yl)oxy)-4-(4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-nitrophenyl)amino)methyl)piperidin-1-yl)ethyl)carbamate 3.** A solution of acid **1** (21.5 mg, 0.0376 mmol) in CH_2Cl_2 (0.4 mL) was treated with sulfonamide **2** (17 mg, 0.038 mmol), DMAP (9.2 mg, 0.075 mmol), and EDCI-HCl (14 mg, 0.075 mmol). The solution was stirred at room temperature for 24 h and subsequently diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$, water, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Purification by chromatography on SiO_2 (2% MeOH/CH_2Cl_2, then 10% MeOH/CH_2Cl_2) afforded **3** (21 mg, 55%) as a yellow solid: R_f 0.44 (10% MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (s, 1 H), 8.88 (d, *J* = 2.3 Hz, 1 H), 8.51 (t, *J* = 5.6 Hz, 1 H), 8.18 (d, *J* = 2.6 Hz, 1 H), 8.13 (dd, *J* = 9.2, 2.3 Hz, 1 H), 7.95 (d, *J* = 9.0 Hz, 1 H), 7.69 (d, *J* = 2.5 Hz, 1 H), 7.45 (t, *J* = 2.8 Hz, 1 H), 7.21 (d, *J* = 8.3 Hz, 2 H), 6.91 (d, *J* = 8.3 **H**z, 2 H), 6.88 (d, J = 9.1 Hz, 1 H), 6.56-6.53 (m, 2 H), 5.99 (d, J = 2.3 Hz, 1 H), 4.99 (bs, 1 H), 3.25 (t, J = 6.2 Hz, 4 H), 3.06 (t, *J* = 5.2 Hz, 4 H) 2.95 (d, *J* = 11.2 Hz, 2 H), 2.74 (s, 2 H), 2.46 (t, *J* = 6.3 Hz, 2 H), 2.20 (t, *J* = 5.0 Hz, 4 H), 2.16 (t, *J* = 6.6 Hz, 2 H), 2.04-1.96 (m, 5 H), 1.79 (d, *J* = 13.0 Hz, 2 H), 1.45 (s, 9 H), 1.41 (t, *J* = 6.6 Hz, 2 H), 0.93 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.1, 159.3, 156.0, 155.7, 147.9, 146.2, 145.3, 142.1, 136.7, 135.5, 135.3, 133.9, 131.9, 130.7, 129.7, 129.1, 128.2, 127.0, 125.3, 120.6, 120.6, 113.7, 109.0, 108.9, 101.6, 100.7, 77.2, 60.3, 57.4, 53.1, 52.2, 49.0, 47.0, 46.9, 35.6, 35.3, 30.2, 29.2, 28.5, 28.2, 25.6; LC-MS (ESI) *m/z* calc for $C_{52}H_{65}ClN_9O_8S$ $[M+H]^+$ 1010.44, found 1010.74.

Note: 1H NMR data correlated with those reported for a similar analog (the *N*-methylpiperidine instead of the derivative reported herein).[2] Note: the peak at 4.99 ppm was not listed in the

assignment of compound **3**. It is conceivable that the coupling reaction actually generated the tertiary amide (coupling through the aniline nitrogen versus the sulfonamide; additional experiments would need to be performed to rule out this possibility. In any case, the biological data reported in this paper correspond to that generated from the compounds with the characterization data reported herein.



2-((1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)oxy)-4-(4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((1-(2-(3-(5,5-difluoro-7,9-dimethyl-5*H*-5λ^4,6λ^4-dipyrrolo[1,2-*c*:2',1'-f][1,3,2]diazaborinin-3-yl)propanamido)ethyl)piperidin-4-yl)methyl)amino)-3-nitrophenyl)sulfonyl)benzamide (ABT199-BODIPY, 8). A solution of **3** (11 mg, 0.011 mmol) in CH_2Cl_2 (1 mL) was treated with Et_3SiH (0.1 mL) and TFA (1 mL). The reaction mixture was stirred for 3 h at room temperature, and the volatiles were removed under a stream of argon. The residue was dissolved in 25% EtOH/EtOAc (30 mL) and washed with saturated aqueous NaH_2PO_4 and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude mixture was coevaporated with toluene (3 x) and carried forward without further purification: LC-MS (ESI) *m/z* calc for $C_{47}H_{57}ClN_9O_6S$ $[M+H]^+$ 910.38, found 910.36.

Note: The mass recovery for this protocol was typically >100% (based on the free amine). The crude material likely exists as a mixture of salts.

To form the BODIPY conjugate, a sample of amine 4 (3.0 mg, 0.0033 mmol, assuming the MW of the free base) was incubated with BODIPY FL, SE ® (Invitrogen) (0.64 mg, 0.0016 mmol) and *i*-Pr_2EtN (1 μL, 0.006 mmol) in anhydrous DMF (165 μL) overnight. The entire solution was loaded directly onto a Biotage 10 g SiO_2 cartridge and purified by chromatography (Biotage, 10 g SiO_2, 0-10% MeOH/CH_2Cl_2; 100 mL at 12 mL/min, then 10% MeOH/CH_2Cl_2, 50 mL at 12 mL/min); the product elutes at ca. 130 mL (10% MeOH/CH_2Cl_2) to afford **8** (1.7 mg, 89%) as a red film: ^{1}H NMR (characteristic signals) (400 MHz, $CDCl_3$) δ 10.04 (b), 8.83 (s), 8.44-8.40 (m), 8.12 (m), 8.07-8.03 (m), 7.89 (d, *J* = 9.0 Hz), 7.64 (bd), 7.47 (s), 7.39 (t, *J* = 2.6 Hz), 7.09-7.04 (m), 6.95 (s), 6.86 (d, *J* =8.4 Hz), 6.77 (d, *J* = 8.4 Hz), 6.50-6.48 (m), 6.25 (d, *J* =

4.2 Hz), 6.05 (s), 5.95 (d, J = 2.1 Hz), 3.24 (t, J = 7.8 Hz), 2.60 (t, J = 7.6 Hz); LC-MS (ESI) *m/z* calc for $C_{61}H_{70}BClF_2N_{11}O_7S$ $[M+H]^+$ 1184.49, found 1184.73.

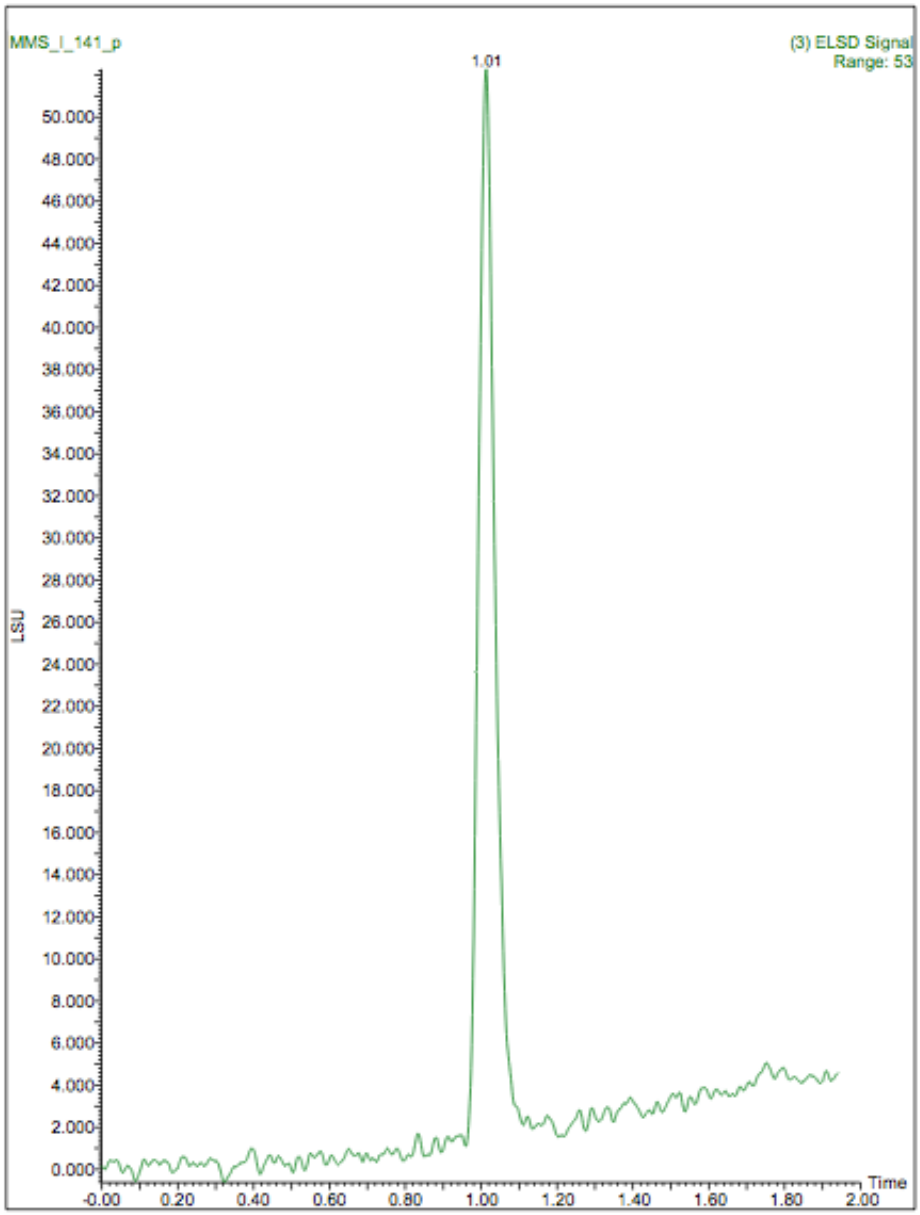
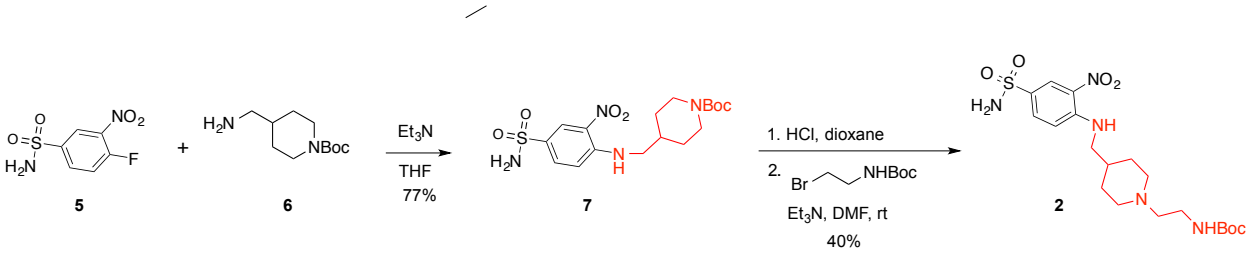


Figure S4. LC-ELSD chromatogram of ABT-199-BODIPY. Chromatography conditions are as follows: Waters XTerra® C18 5 µm column was used (eluents 0.1% formic acid (v/ v) in water and MeCN; gradient: 0–1.5 min, 5–100% then 1.5–2.0 min 100%. More detail is provided in Materials and Instrumentation (p. 1).

Scheme S4. Synthesis of sulfonamide



***tert*-Butyl 4-(((2-nitro-4-sulfamoylphenyl)amino)methyl)piperidine-1-carboxylate 7** An oven-dried reaction vessel was charged with 4-fluoro-3-nitrobenzenesulfonamide (115 mg, 0.522 mmol), THF (1.8 mL), 1-*N*-Boc-4-methylaminopiperidine (112 mg, 0.464 mmol), and Et_3N (72 μL, 0.52 mmol). The bright yellow suspension was stirred at room temperature overnight and subsequently acidified with 3 M aqueous HCl. The mixture was diluted with ether and water, and the ethereal layer was washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated in vacuo to afford **7** (166 mg, 77%) as a yellow solid. The material was carried forward to the next step without further purification. 1H NMR (400 MHz, DMSO-d6) δ 8.56 (t, J = 6.3 Hz, 1 H), 8.47 (d, J = 3.3 Hz, 1 H), 7.83-7.81(m, 1 H), 7.31-7.28 (m, 3 H), 3.95 (bd, J = 2.0 Hz, 2 H) 3.36 (t, J = 6.6 Hz, 2 H), 2.69-2.64 (m, 2 H), 1.87-1.81 (m, 1 H), 1.68 (d, J = 12.7 Hz, 2 H) *or* 1.70-1.66 (m, 2 H), 1.39 (s, 9 H), 1.44 (dddd J = 12.2, 12.2, 12.2, 4.2 Hz); LC-MS (ESI) calc for $C_{17}H_{26}N_4NaO_6S$ $[M+Na]^+$ 437.15, found 437.25.

***tert*-Butyl (2-(4-(((2-nitro-4-sulfamoylphenyl)amino)methyl)piperidin-1-yl)ethyl)carbamate 2**. A solution of **7** (100 mg, 0.241 mmol) in 4 M HCl in dioxanes (3.3 mL) was stirred at room temperature for 2 h. The solvent was removed *in vacuo*, and the residue was dissolved in DMF (2.4 mL). Et_3N (57 μL, 0.41 mmol) and *tert*-butyl (2-bromoethyl)carbamate (59 mg, 0.27 mmol) were added, and the solution was stirred at room temperature overnight. The solvent was removed *in vacuo*, and the residue was purified by chromatography using a Biotage system (10 g SiO_2 cartridge; linear gradient of 0-20% MeOH/CH_2Cl_2 over 10 min) to afford **2** (44 mg, 40%) as a yellow solid: R_f 0.29 (CH_2Cl_2/acetone/Et_3N, 5:4:1); ^{1}H NMR (characteristic signals) (400 MHz, DMSO-d_6) δ 8.55 (t, J = 5.8 Hz, 1 H), 8.47 (d, J = 2.1 Hz, 1 H), 7.83-7.81 (m, 1 H), 7.31 (s, 2 H), 7.28 (d, J = 9.2 Hz, 1 H), 6.60 (s, 1 H), 1.37 (s, 9 H); LC-MS (ESI) *m/z* calc for $C_{19}H_{32}N_5O_6S$ $[M+H]^+$ 458.21, found 458.33.

In vitro competitive inhibition. The ELISA-based assay was carried out according to a previously published protocol.[7] Biotinylated Bim peptide (residues 81-106: (Biotin)-β-Ala-β-Asp-Met-arg-Pro-Glu-Ile-Trp-Ile-Ala-Gln-Glu-Leu-Arg-Arg-Ile-Gly-Asp-Glu-Phe-Asn-Ala-Tyr-Tyr-Ala-Arg-Arg-NH2) was purchased from Tocris/R&D Biosciences (catalog no. 3526). A stock solution (0.18 μg/mL) of biotinylated Bim peptide was prepared by dissolving 3.6 μL of a 137 μM DMSO stock solution in Superblock Blocking Buffer (Thermo Scientific), followed by a 10-fold dilution in Superblock Blocking Buffer. The solution (100 μL/well) was applied to a streptavidin coated 96-well plate (Pierce® Streptavidin Coated High Binding Capacity plates, Thermo Scientific catalog no. 15500) and incubated at room temperature with shaking for 1.5 h to allow for Bim-Biotin-Streptavidin complexation.

Different concentrations of inhibitors (prepared by serial dilution of 1 mM DMSO stocks in 1X PBS, normalized to 0.1% DMSO) were incubated with 20 nM purified His-tagged Bcl-2 protein (R&D Systems) for 1 h at room temperature (150 μL of each inhibitor/protein solution were prepared).

The Bim-Biotin-Streptavidin coated plate was washed with 0.05% Tween-20 in PBS (3 x 5 min), and 100 μL aliquots of the inhibitor/protein solutions were transferred to the Bim-Biotin-Streptavidin coated plate and incubated at room temperature for 2 h. The plate was washed with 0.05% Tween-20 in PBS (3 x 5 min), and His-Tag (27E8) mouse mAb-HRP conjugate (Cell Signaling Technology, catalog no. 9991S) (1:250 dilution in Superblock Blocking Buffer) was applied to the wells, and the plate was incubated for 1 h at room temperature. The plate was washed with 0.05% Tween-20 in PBS (3 x 5 min), and a solution of *o*-phenylenediamine (prepared by dissolving one pre-packaged tablets (Sigma-Aldrich P5412) in 50 mL pH 5.0 0.2 M Na_2HPO_4/0.1 M citric acid buffer and adding 30 μL of 30% H_2O_2) was applied to the wells. The enzymatic activity was detected by measuring the absorbance at 450 nm over 40 min (measuring every 30 s) on a TECAN Safire2 plate reader. Bcl-2 protein binding was assessed by comparing the relative rates of the enzymatic reaction; values were normalized by extending the assay range to a 100% scale as determined by the average of wells at each individual drug concentration. After log-transforming the inhibitor concentrations, IC50 values were determined after fitting curves by a 4-parameter logistic curve using Graphpad Prism Software.

Cell Culture. Cells were cultured in an incubator at 37 ºC with 5% CO_2. All cell lines were grown with a base medium supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin. Respective base mediums for each cell line were: RPMI: 4T1, OVCA-429; DMEM: Panc-1, A431, HT-1080, MCF-7, and MDA-MB-231. All cell lines were acquired from ATCC.

mCherry-Hyg-C1 was constructed by restriction digest of mCherry-Parkin (Addgene) and pAcGFP1-Hyg-C1 (Clontech) with NheI and BglII. pAcGFP1 was then replaced with mCherry by ligation of mCherry into the Hyg-C1 vector backbone. mCherry-Hyg-C1 and pEMD-Bcl2 (Addgene) were digested with BglII and KpnI followed by ligation of Bcl2 into mCherry-Hyg-C1 to make mCherry-Bcl2. The mCherry-Bcl2 insert was sequenced in its entirety. MDA-MB-231 cells were transfected with the mCherry-Bcl2 plasmid using Lipofectamine 2000 (Life Technologies), according to the manufacturers instructions. Cells were selected in 300 ug/ml hygromycin and single fluorescent clones were isolated and maintained in 40 μg/ml hygromycin.

Cell viability. Cell viability assays were performed in 96-well format; cells were seeded at 40,000 cells/well in 200 μL RPMI 24 hours prior to application of the test compounds. Increasing concentrations of inhibitors (1 nM to 10 μM) were applied to each well using an HP D300 Digital Dispenser (from initial compound stocks at 5 or 10 μM)—all wells were normalized to 0.1% DMSO. After incubating for 48 h, cell viability was quantified using the PrestoBlue cell viability reagent (Life Technologies, catalog #A-13261, Grand Island, NY) and the TECAN Safire2 plate reader (Mannedorf, Switzerland). Fluorescence measurements were normalized to cells exposed to a DMSO control (no inhibitor), and drug concentrations were log-transformed, and half-maximal effective concentrations were estimated by nonlinear regression to a sigmoidal dose response using the Prism 6.0 Software Suite (GraphPad, La Jolla, CA). All measurements were preformed in triplicate and are presented as means +/- 95% confidence intervals.

In addition to Bcl-2 dependent cell lines shown in the main text, we also tested the efficacy of ABT-199 and ABT-199-BODIPY against non-Bcl-2 dependent cell lines including OVCA-429, which showed an IC50 of > 10 μM. Data for both ABT-199 and ABT-199-BODIPY up to concentrations used for imaging are shown in Figure S5.

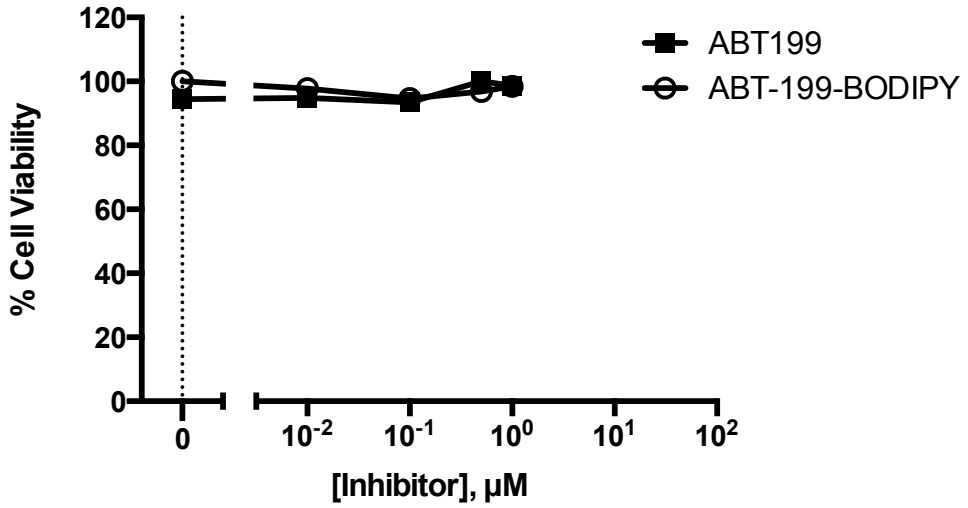


Figure S5. Cell viability analysis for ABT-199 and ABT-199-BODIPY in OVCA-429 cells.

Live Cell Imaging: Live cell microscopy was carried out using a DeltaVision imaging system (Applied Precision), which consists of an environmental chamber heated to 37 °C, with CO_2 bubbled through a water bath, an automated Olympus IX70 inverted microscope and a CoolSnap HQ2 CCD camera. BODIPY-FL was imaged using a filter cube with 490/20 excitation-525/30 emission while RFP was imaged using a filter cube with 555/28 excitation-605/52 emission. Cells were grown and imaged in 96-well glass bottom plates (Greiner BioOne). Prior to imaging, cells were washed 3X 5 minutes in pre-warmed cell media.

Fixed Cell Imaging: Cells were cultured on Millicell Well EZ well glass slides and grown for 48 hours. Cells were then treated with 1 μM concentrations of ABT-199-BODIPY overnight. Following treatment, cells were washed 3X with PBS and then fixed in a solution of 4% Paraformaldahyde in PBS and fixed at room temperature for 20 minutes. Following fixation, slides were washed 3X in PBS, slide chambers were removed, and slides were mounted using mounting media containing DAPI (Vector Laboratories). Slides were then imaged using a customized Olympus FV1000 BX61-W1 (Olympus America) confocal/multiphoton microscope with a programmable, motorized X-Y-Z stage. The objectives used were a 20× UPlanFL (NA=0.50, water), and 60× LUMFL N (NA=1.10, water). DAPI and BODIPY-FL were excited using a 405 nm and 488 nm line of an argon ion laser, respectively. Emitted light was separated using a 405/488/559/635nm dichroic beam splitter and the signal refined by the excitation band pass filters BA505-540 and BA575-605, respectively.

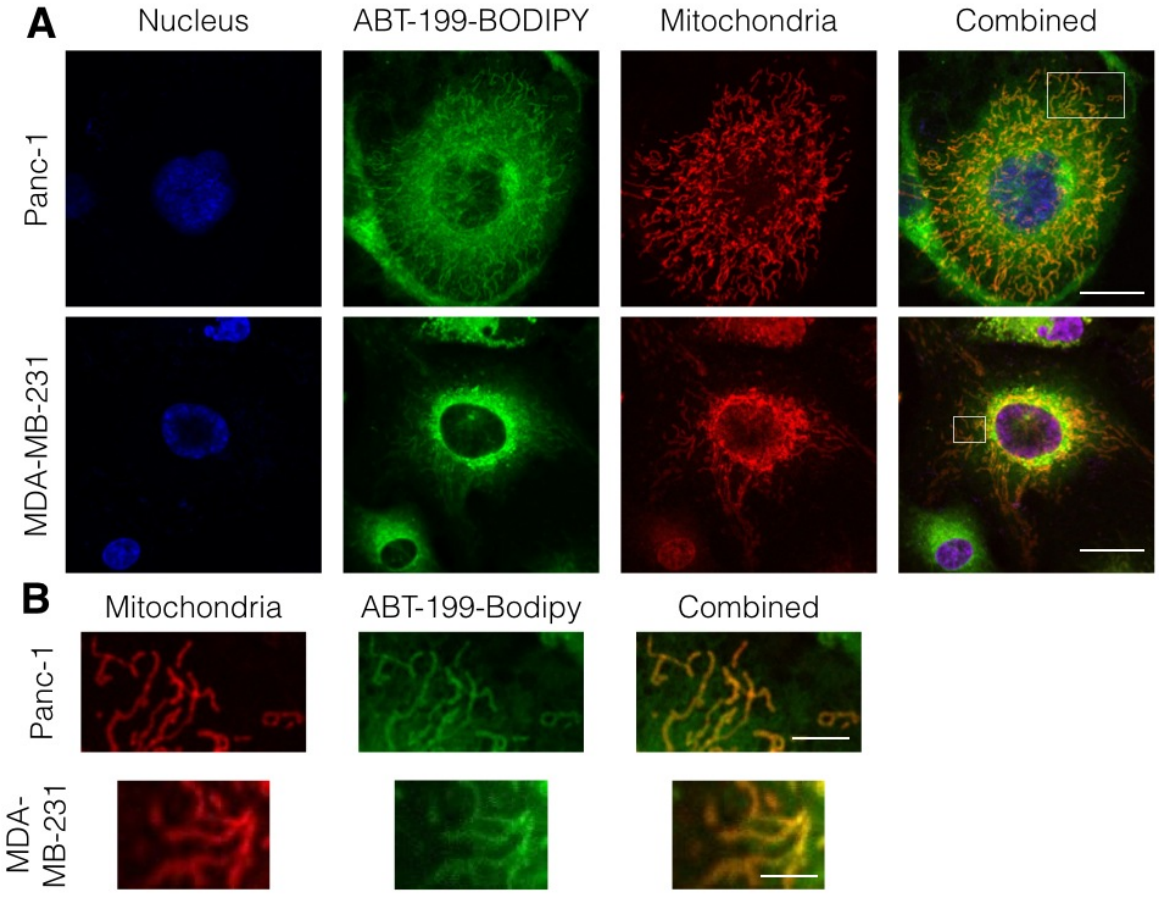


Figure S6. A. Single Cell ABT-199-BODIPY (Green) Colocalization with RFP labeled mitochondria and DAPI (blue) labeled nuclei for Panc-1 and MDA-MB-231 Cells. Scale bars represent 26 μm. B. Zoomed view of peripheral mitochondria (Red) and ABT-199-BODIPY (green) for PANC-1 and MDA-MB-231 cells. Scale bar represents 5 μm for PANC-1 and 0.5 μm for MDA-MB-231 cells.

Immunocytochemistry was performed on treated cells fixed as previously described. Briefly, fixed cells were permeablized with 0.1% Tween-20 in PBS for 20 minutes on a rocker. Cells were then blocked using SuperBlock T20 (TBS) Blocking Buffer (#37536, Thermo Scientific) according to the manufacturers instructions. Cells were then stained with mitochondrial targeted primary antibodies (TOMM-20, Abcam, ab56784) via incubation for 1 hour at room temperature followed by 1 hr. incubation with a secondary antibody (Alexa Fluor 647) for 1 hour at room temperature.

Alternatively, mitochondrial colocalization of ABT-199-BODIPY was imaged by use of RFP expressing genetic reagents. Briefly, 48 hours before fixation cell lines were treated with Cell Lights Mitochondrial RFP Baculovirus (Invitrogen) to create Red Fluorescent Mitochondria in a sub-population of cells (~ 60% efficient across all cells in each cell line). Cells expressing mitochondrial RFP were then fixed and imaged as previously described to observe colocalization of drug.

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Figure S7. ^{1}H NMR Spectrum of **S1**, recorded at 400 MHz in $CDCl_3$.

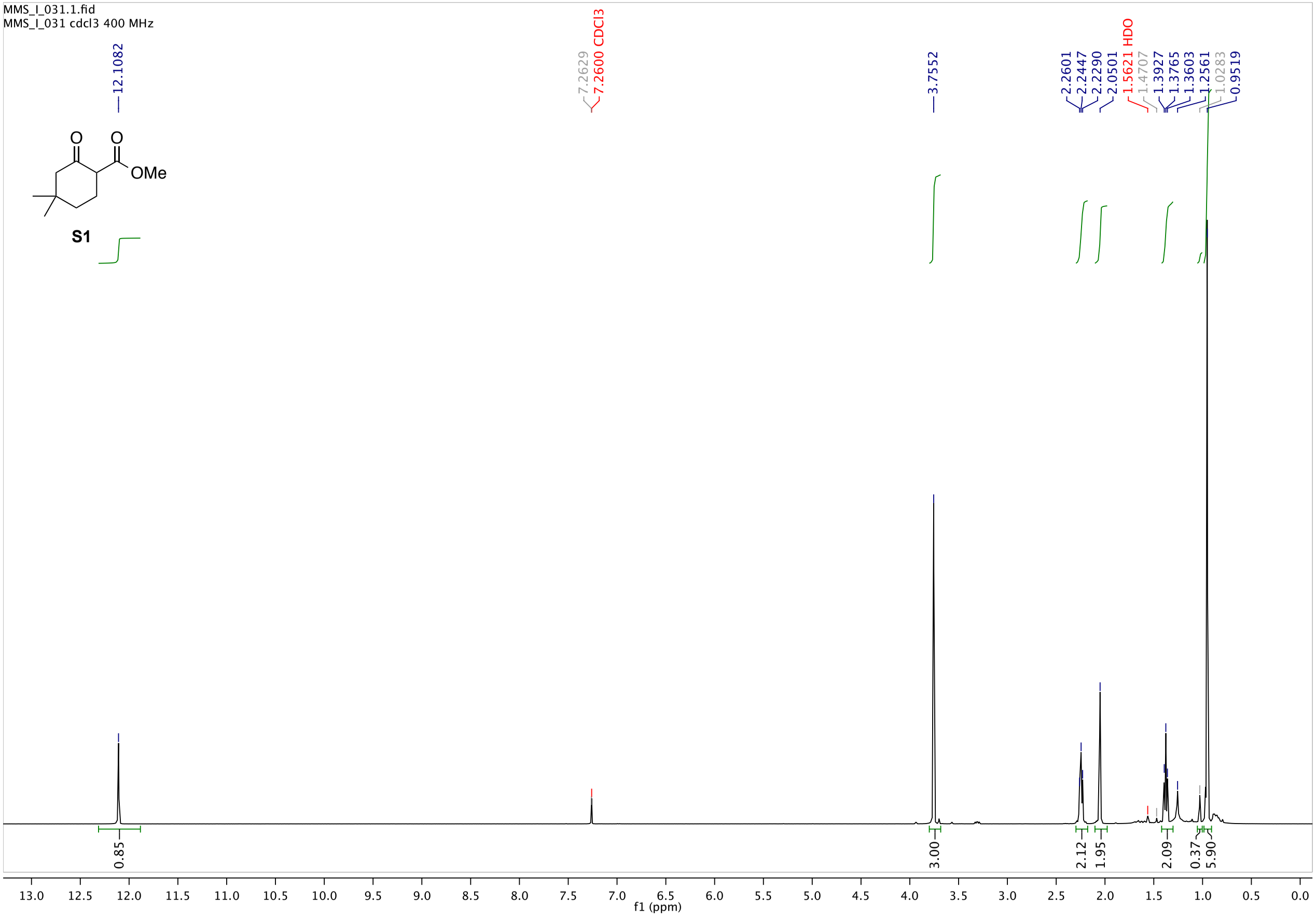


Figure S8. ^{13}C NMR Spectrum of **S1**, recorded at 100 MHz in $CDCl_3$.

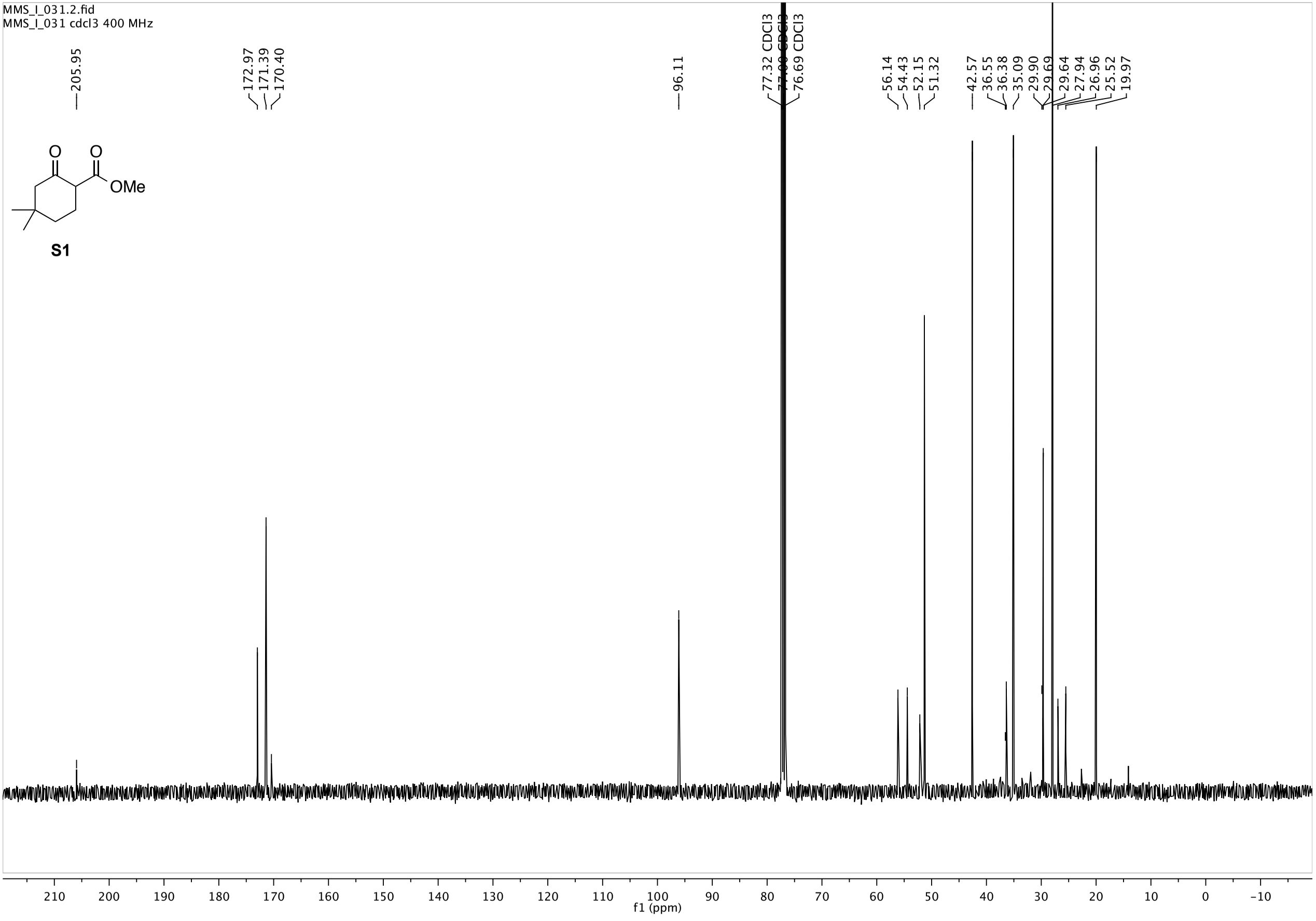


Figure S9. ^{1}H NMR Spectrum of **S2**, recorded at 400 MHz in $CDCl_3$.

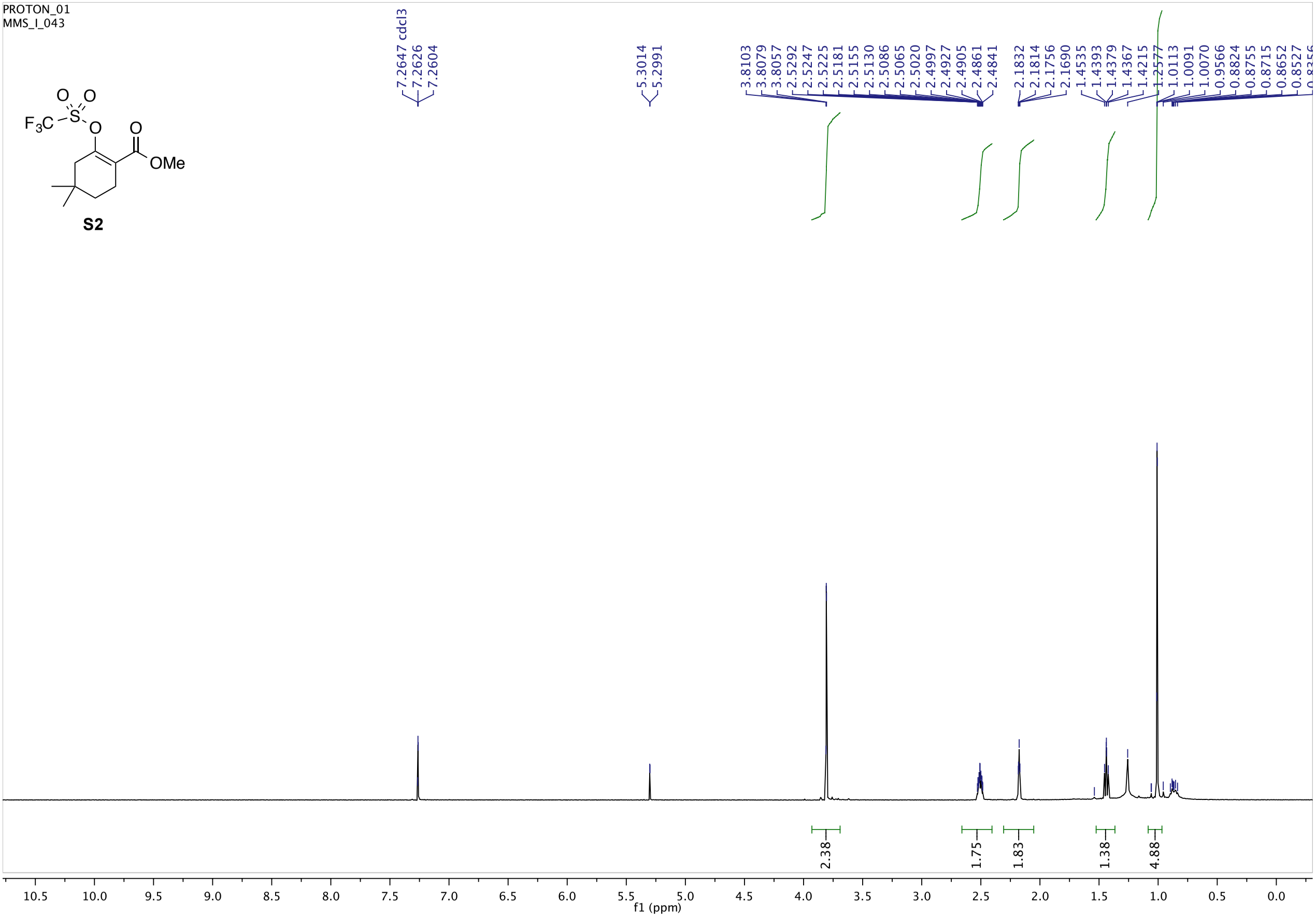


Figure S10. ^{1}H NMR Spectrum of **S4**, recorded at 400 MHz in $CDCl_3$.

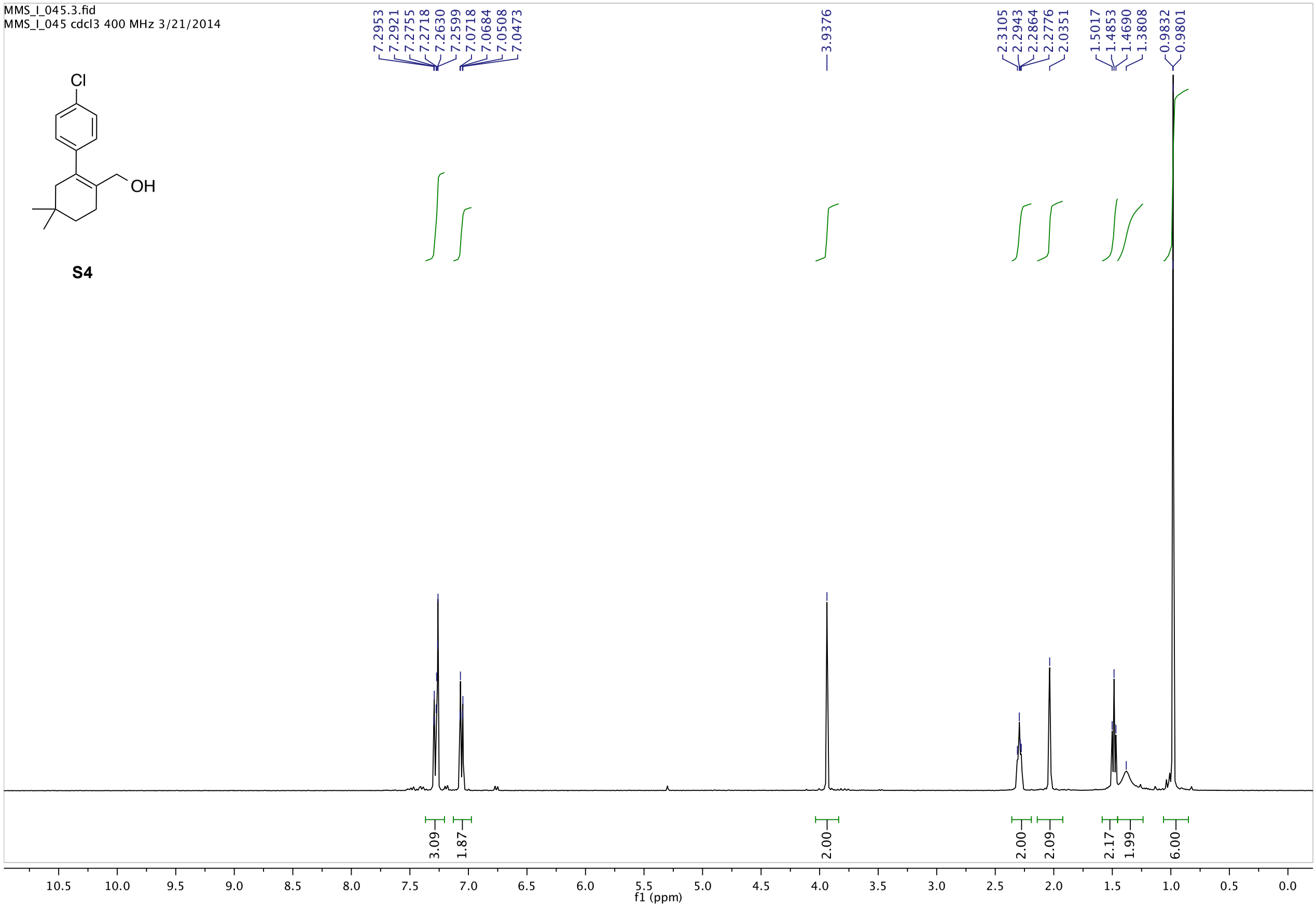


Figure S11. ^{13}C NMR Spectrum of **S4**, recorded at 100 MHz in $CDCl_3$.

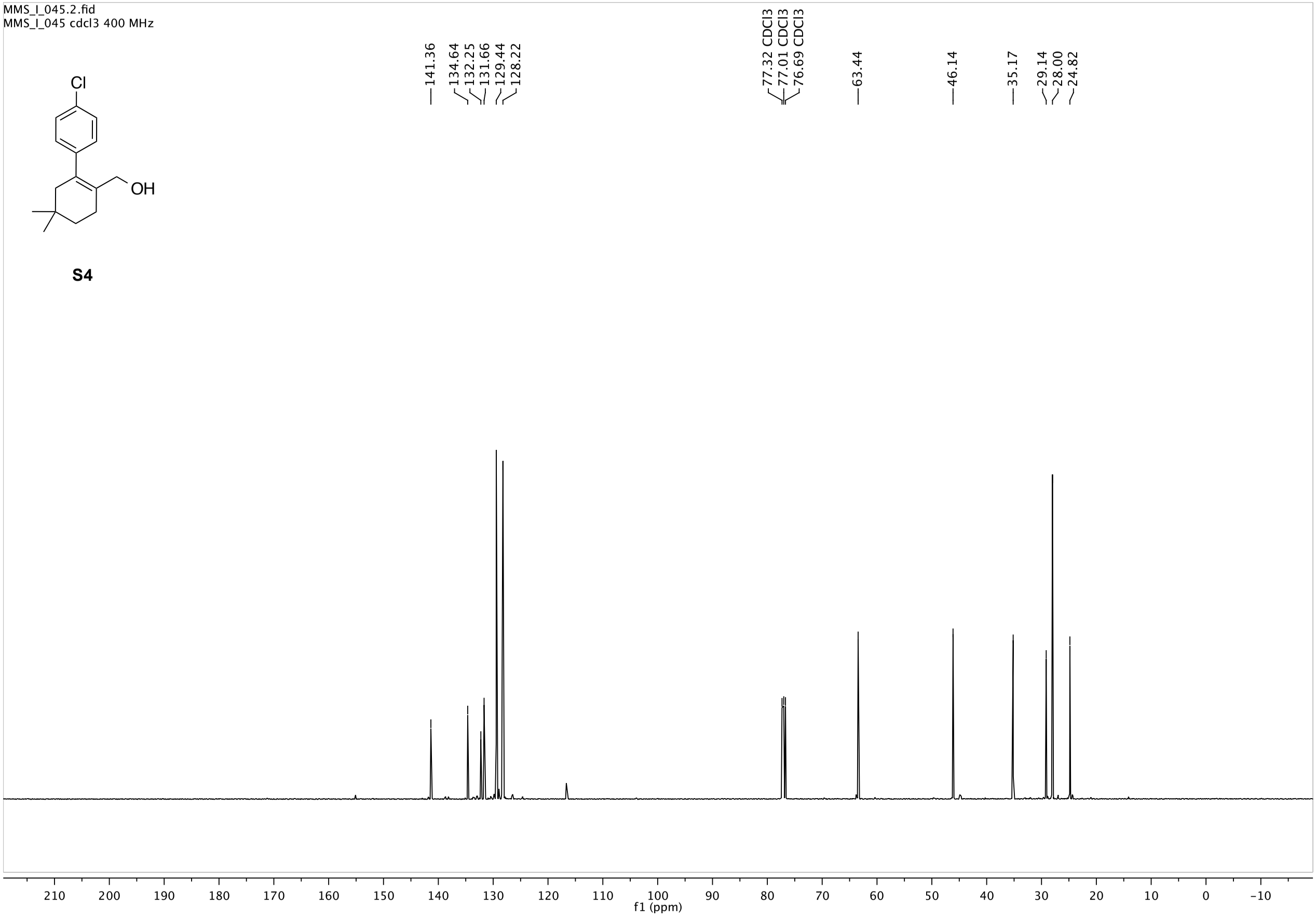


Figure S12. ^{1}H NMR Spectrum of **S5**, recorded at 400 MHz in $CDCl_3$.

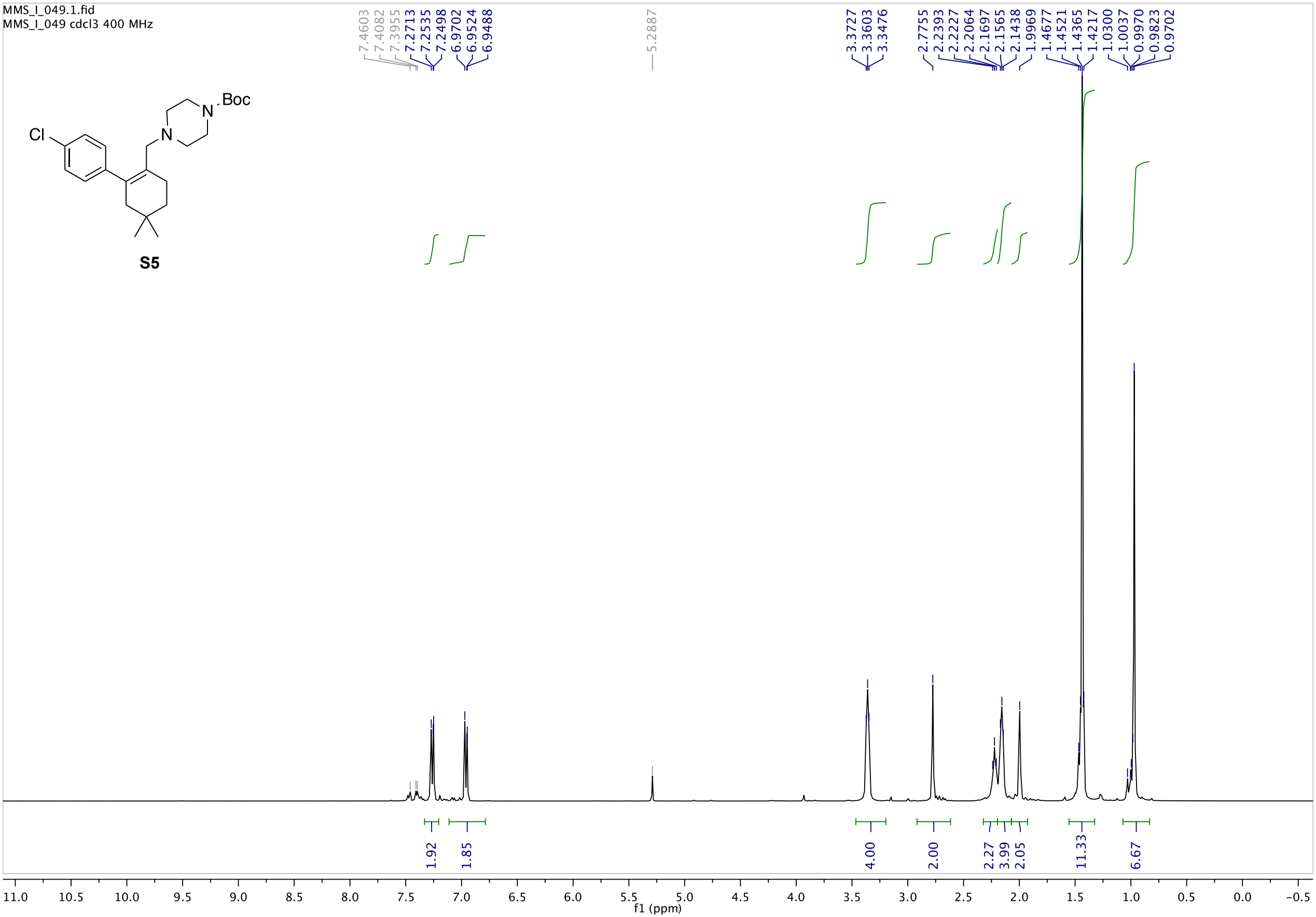


Figure S13. ^{13}C NMR Spectrum of **S5**, recorded at 100 MHz in $CDCl_3$.

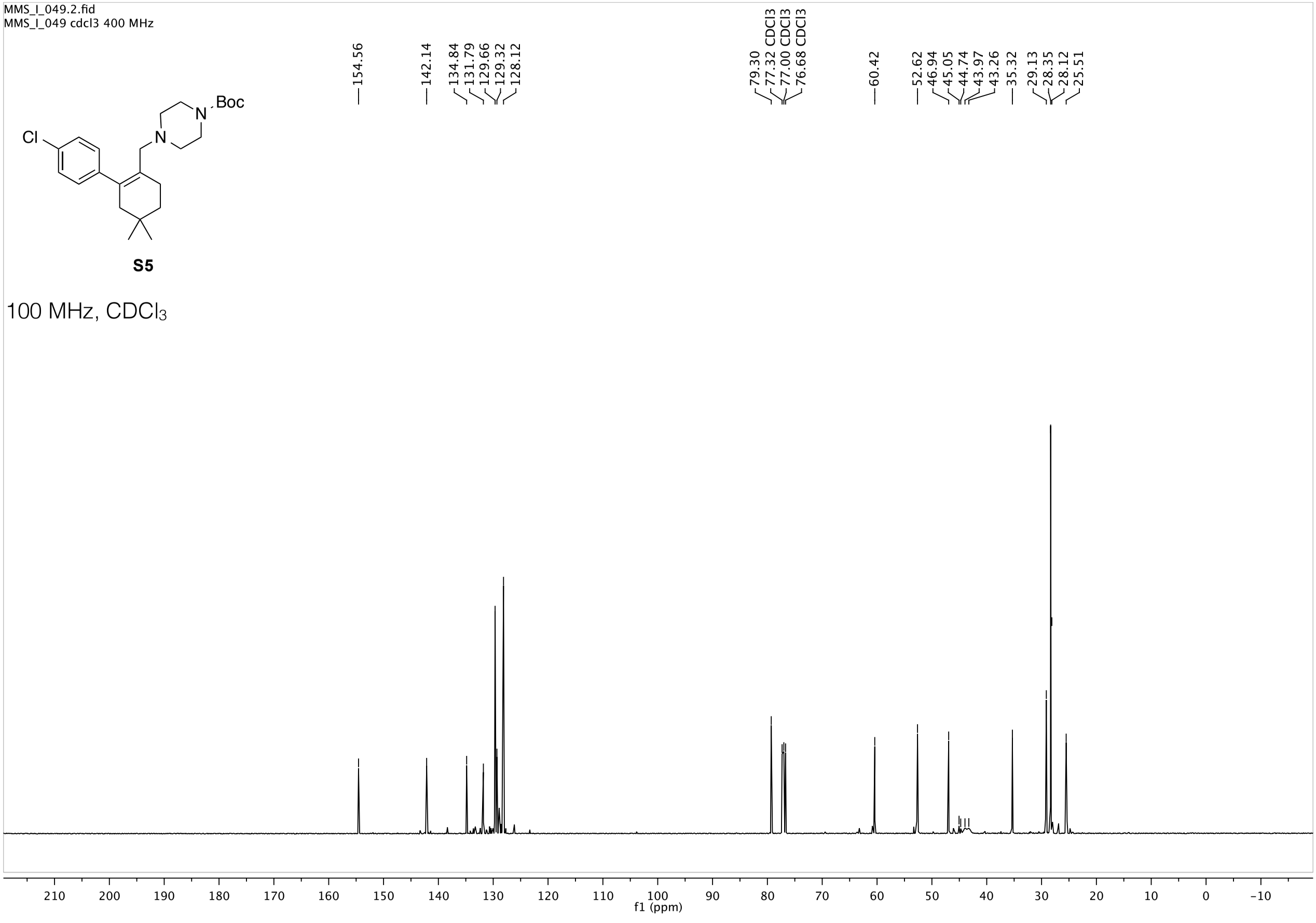


Figure S14. ^{1}H NMR Spectrum of **S7**, recorded at 400 MHz in $CDCl_3$.

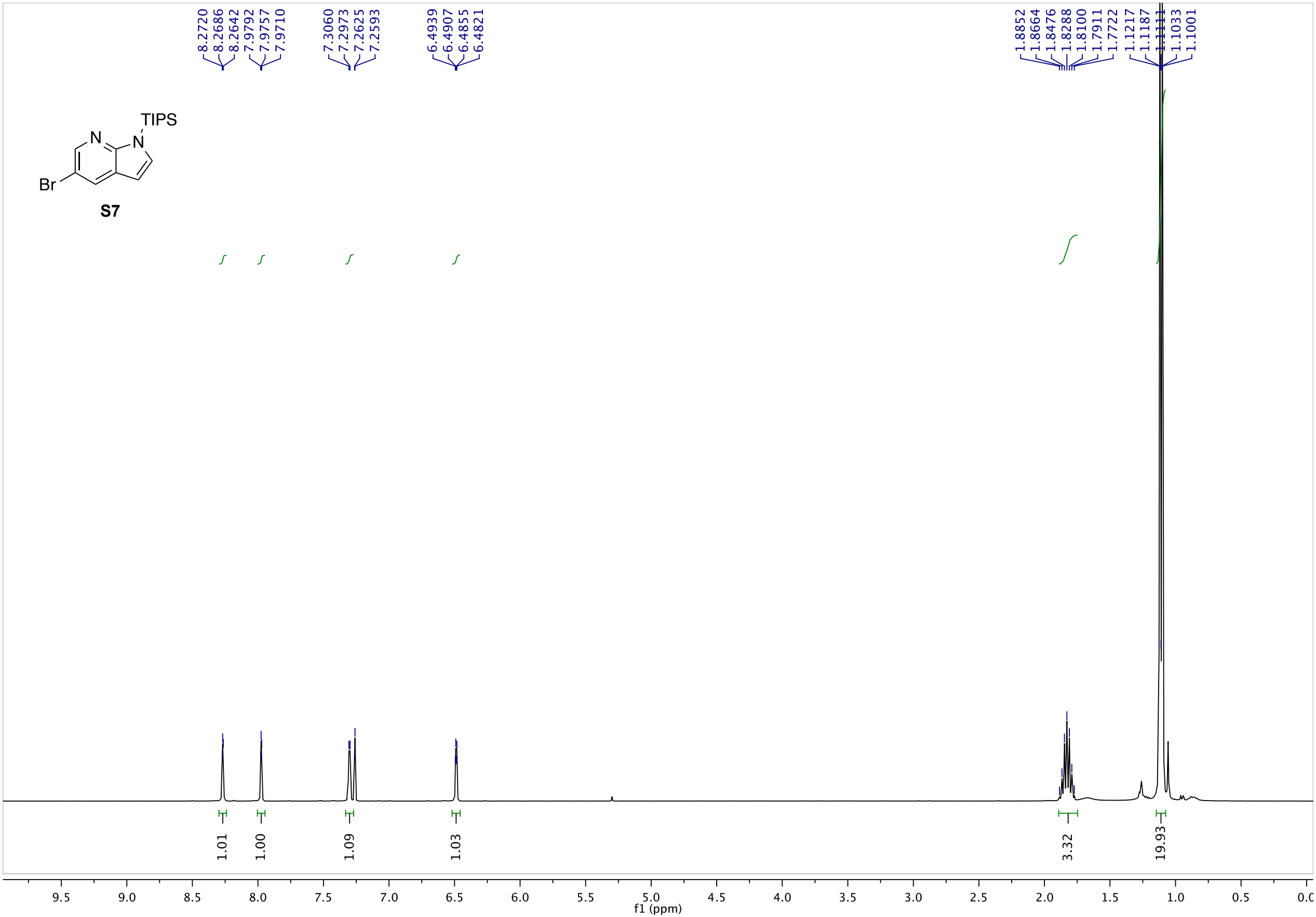


Figure S15. ^{13}C NMR Spectrum of **S7**, recorded at 100 MHz in $CDCl_3$.

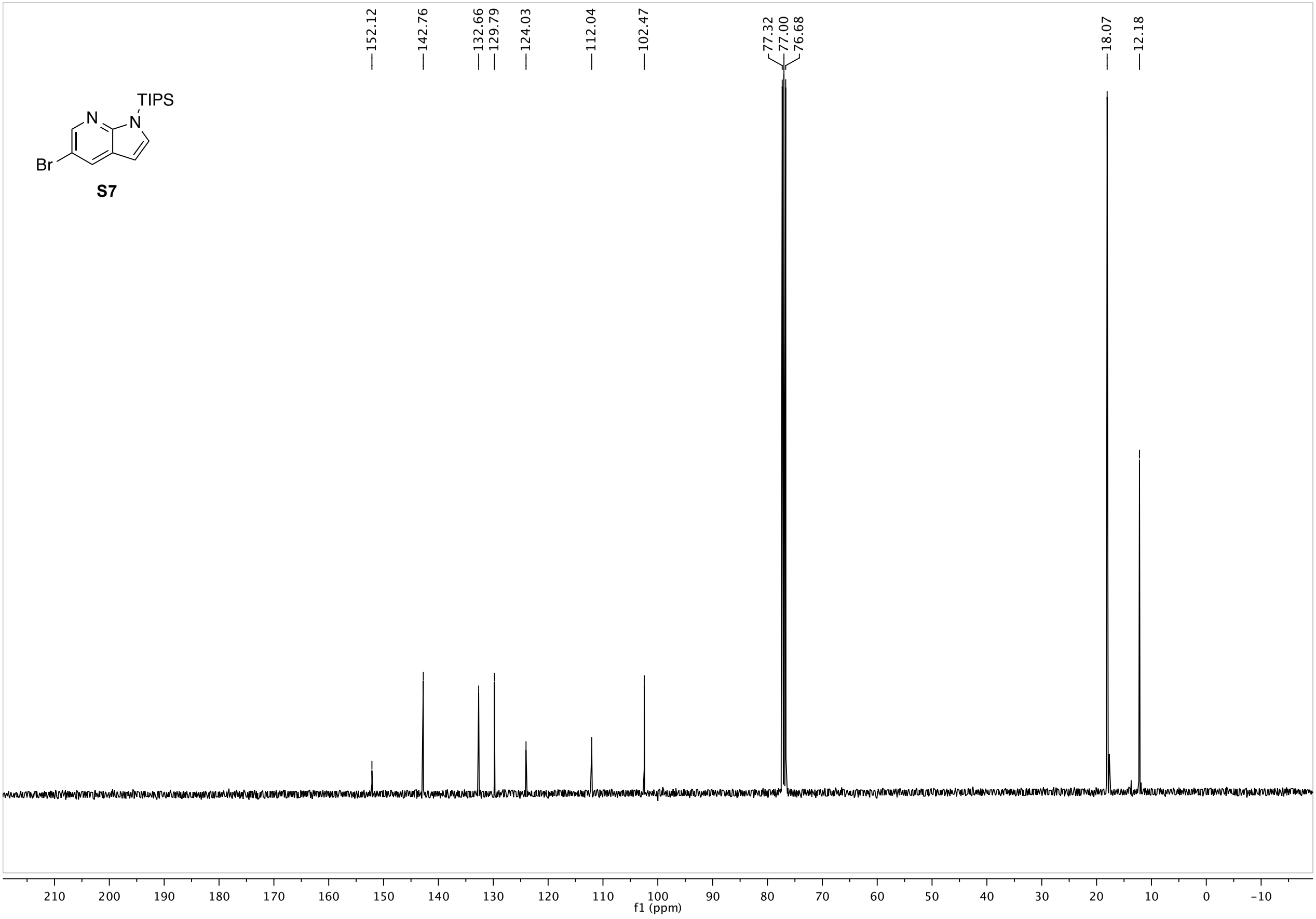


Figure S16. ^{1}H NMR Spectrum of **S8**, recorded at 400 MHz in $CDCl_3$.

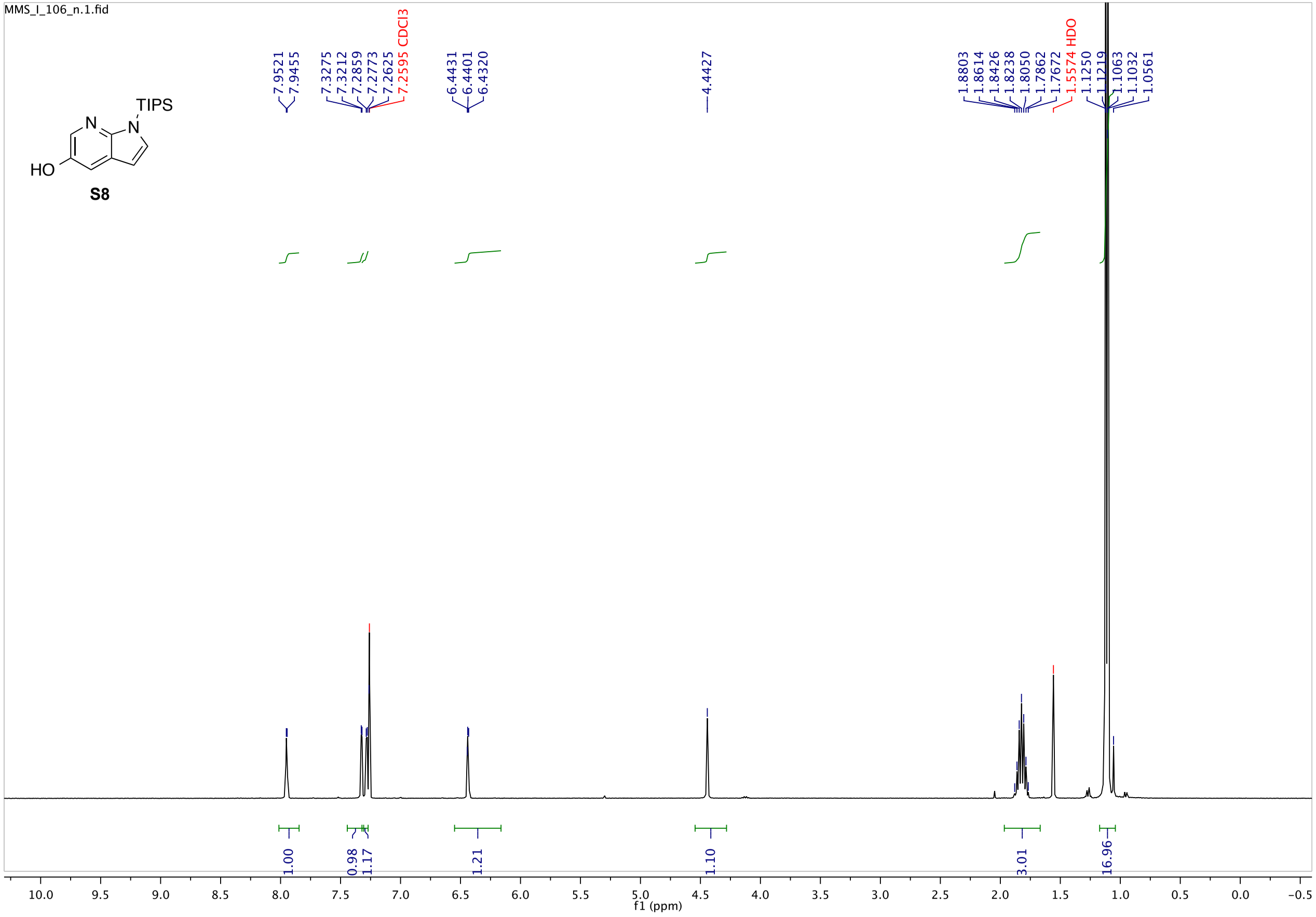


Figure S17. ^{13}C NMR Spectrum of **S8**, recorded at 100 MHz in $CDCl_3$.

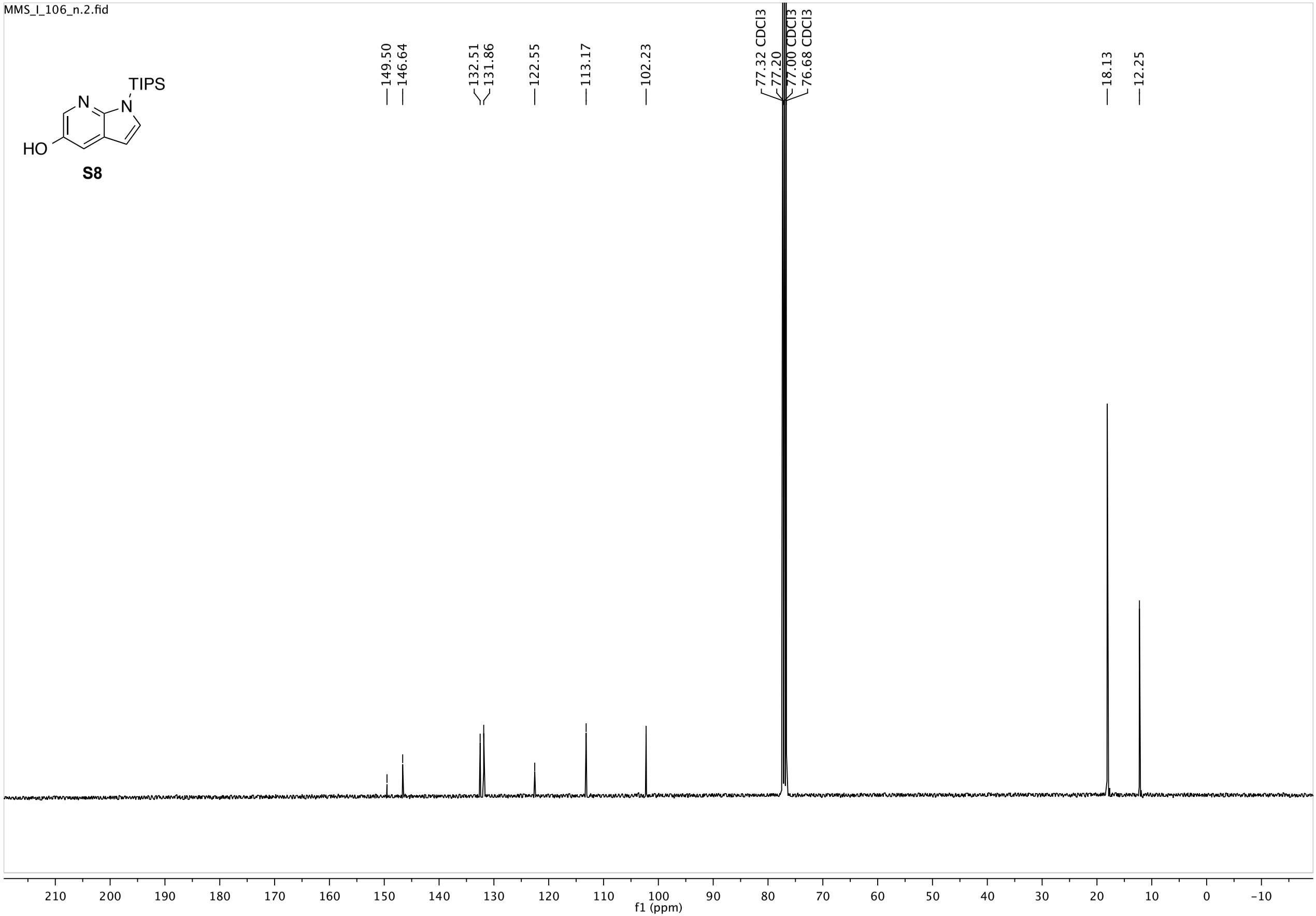


Figure S18. ^{1}H NMR Spectrum of **S8**, recorded at 400 MHz in DMSO-d_6.

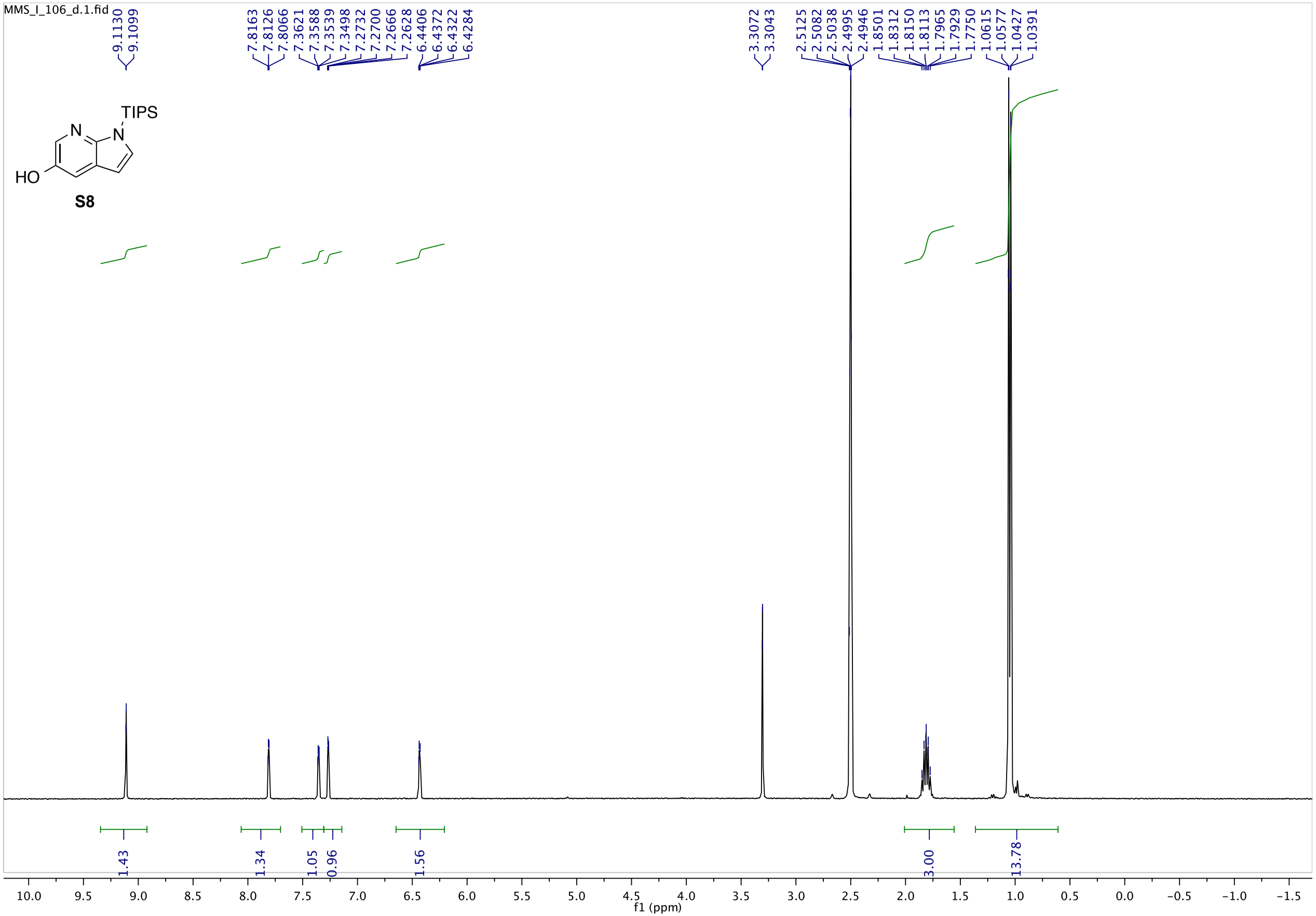


Figure S19. ^{1}H NMR Spectrum of **S9**, recorded at 400 MHz in $CDCl_3$.

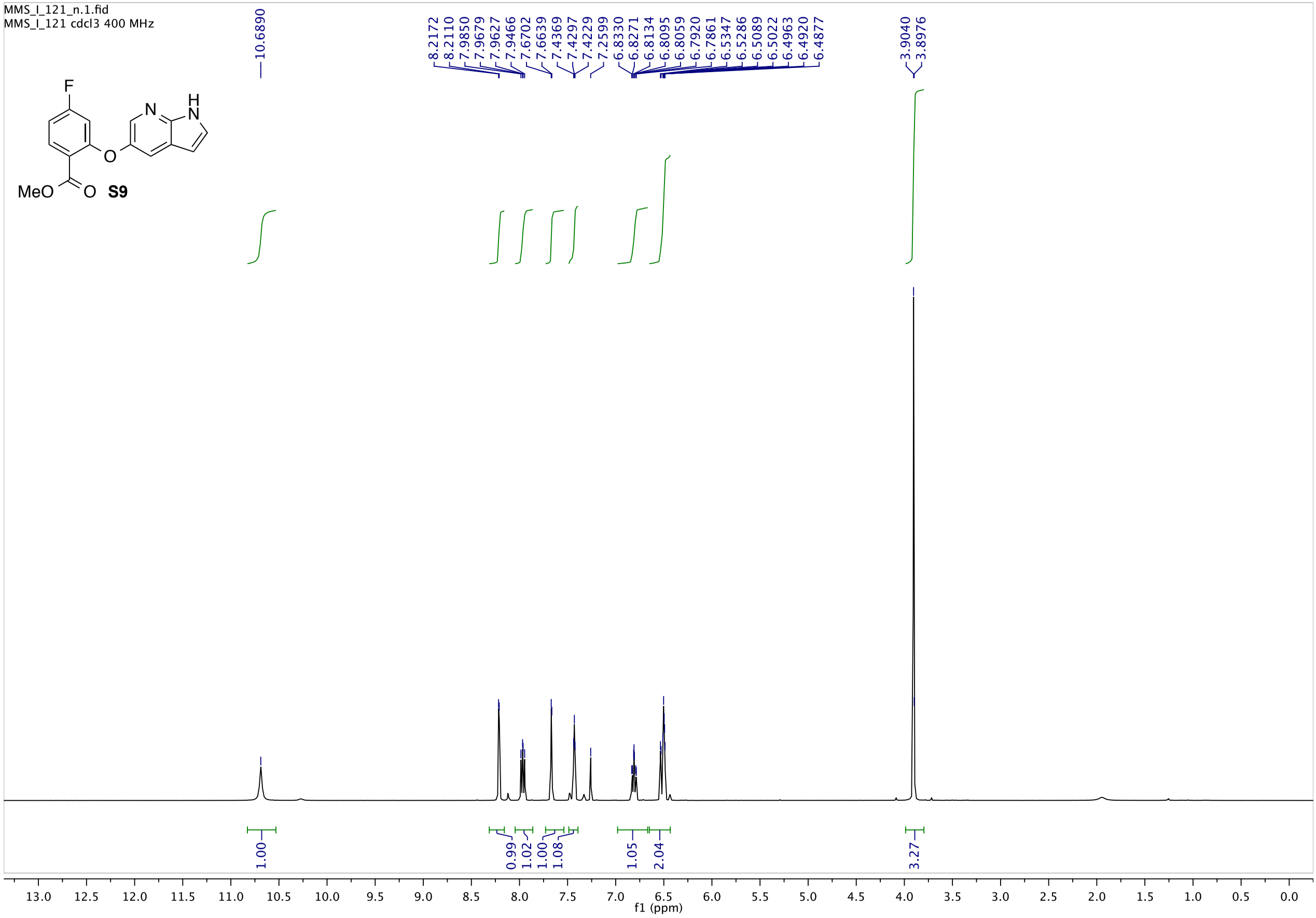


Figure S20. ^{13}C NMR Spectrum of **S9**, recorded at 100 MHz in $CDCl_3$.

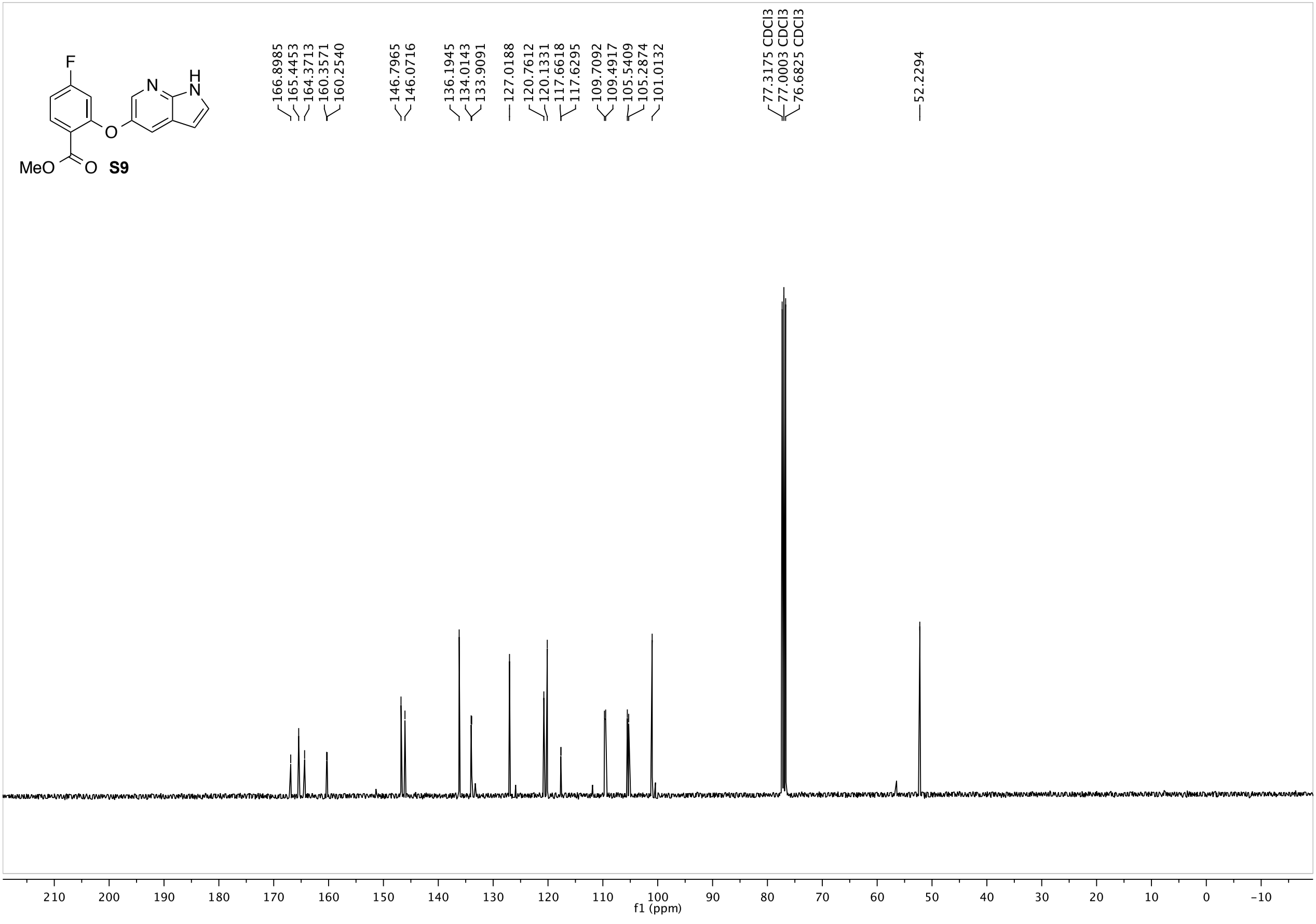
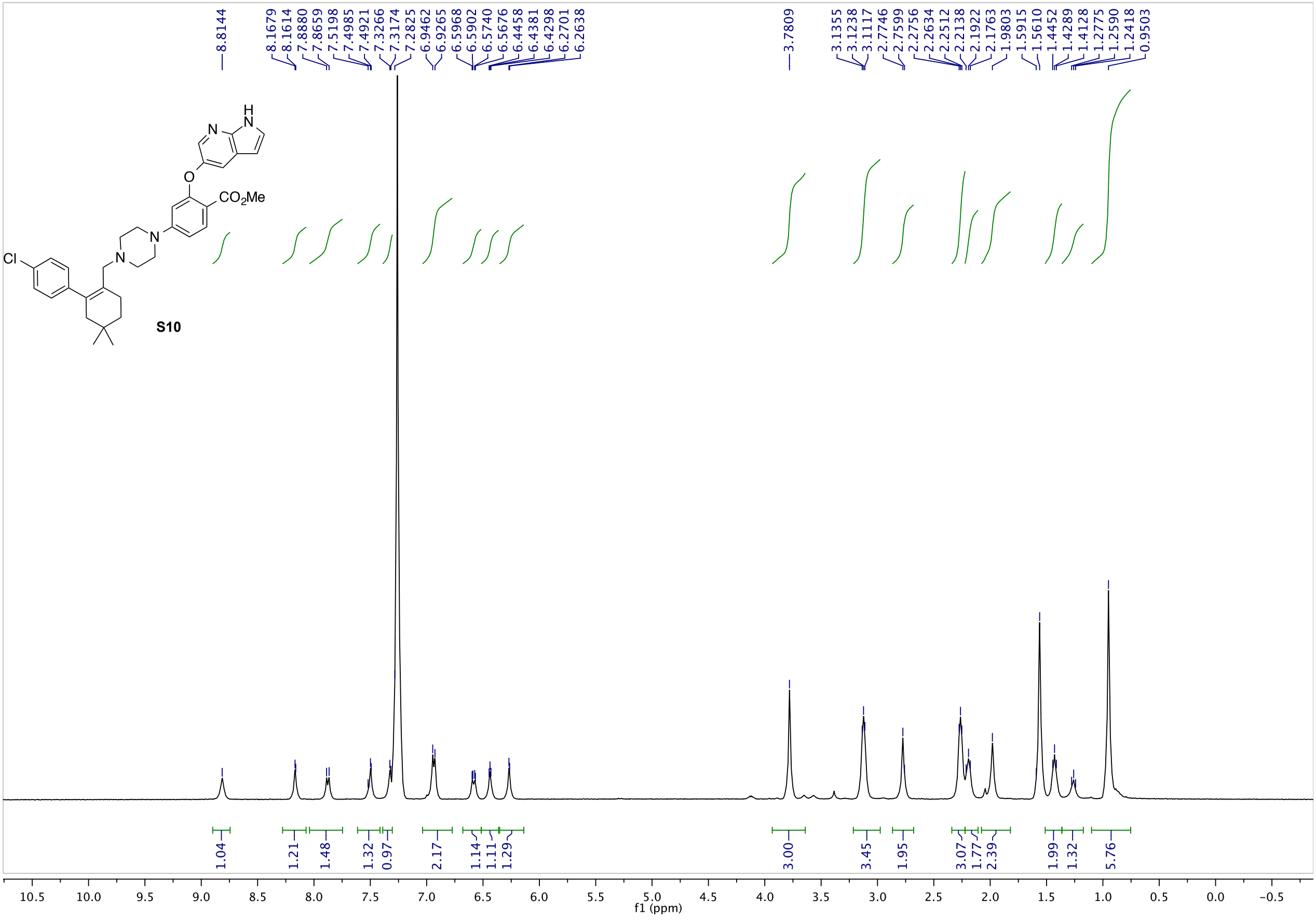


Figure S21. ^{1}H NMR Spectrum of **S10**, recorded at 400 MHz in $CDCl_3$.



S10

Figure S22. ^{1}H NMR Spectrum of **1**, recorded at 400 MHz in $CDCl_3$.

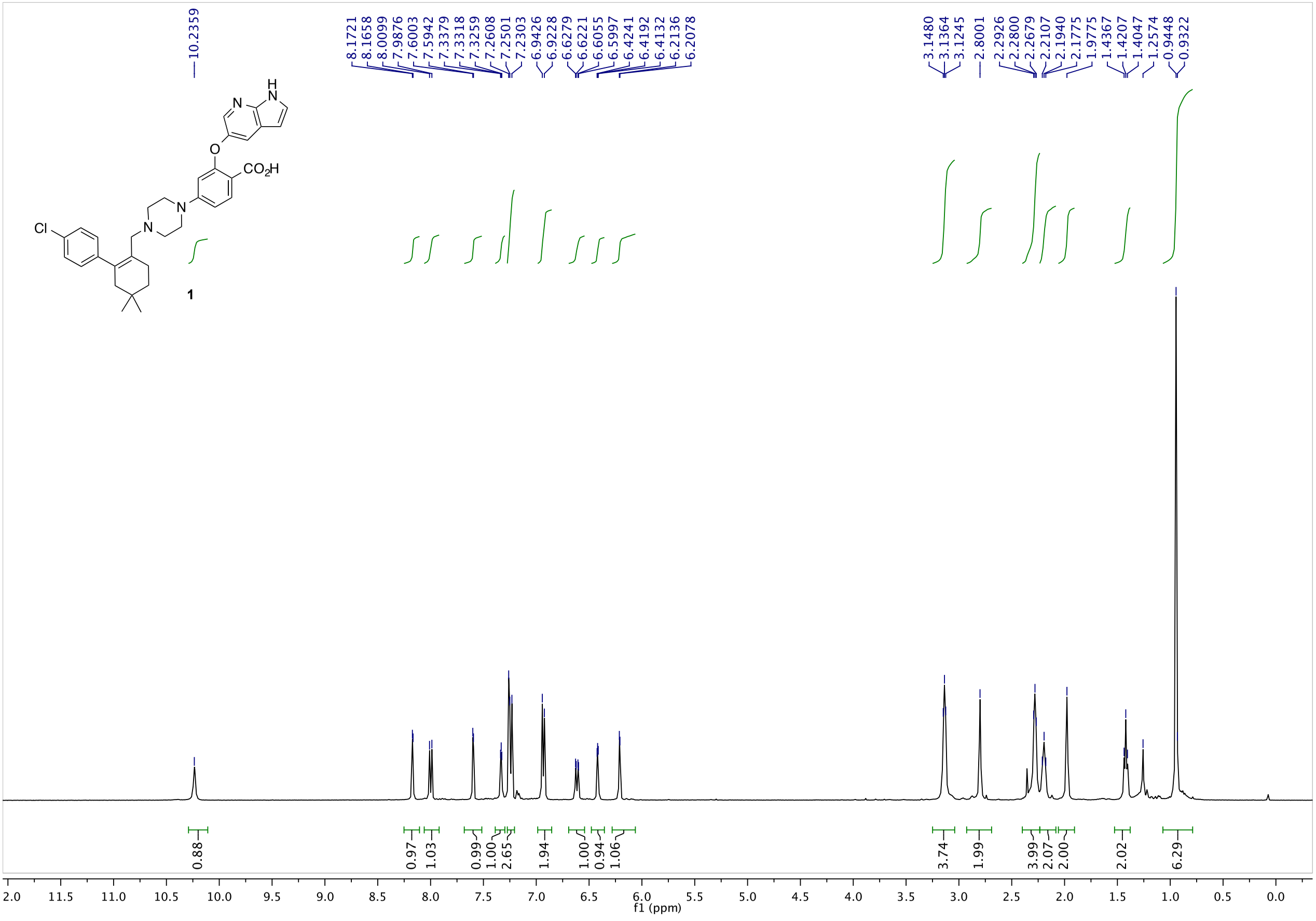


Figure S23. ^{13}C NMR Spectrum of **1**, recorded at 100 MHz in $CDCl_3$.

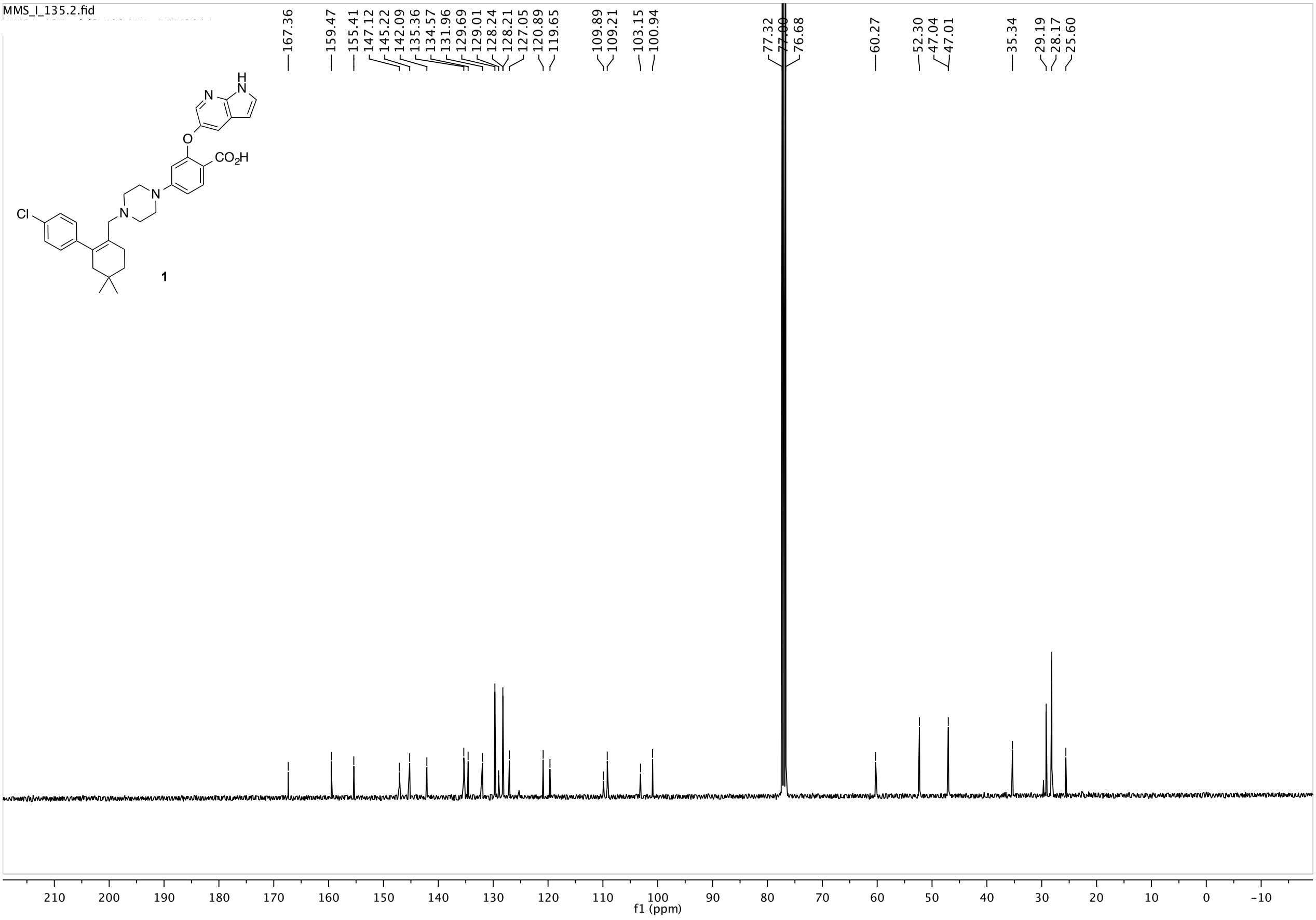


Figure S24. ^{1}H NMR Spectrum of **3**, recorded at 400 MHz in $CDCl_3$.

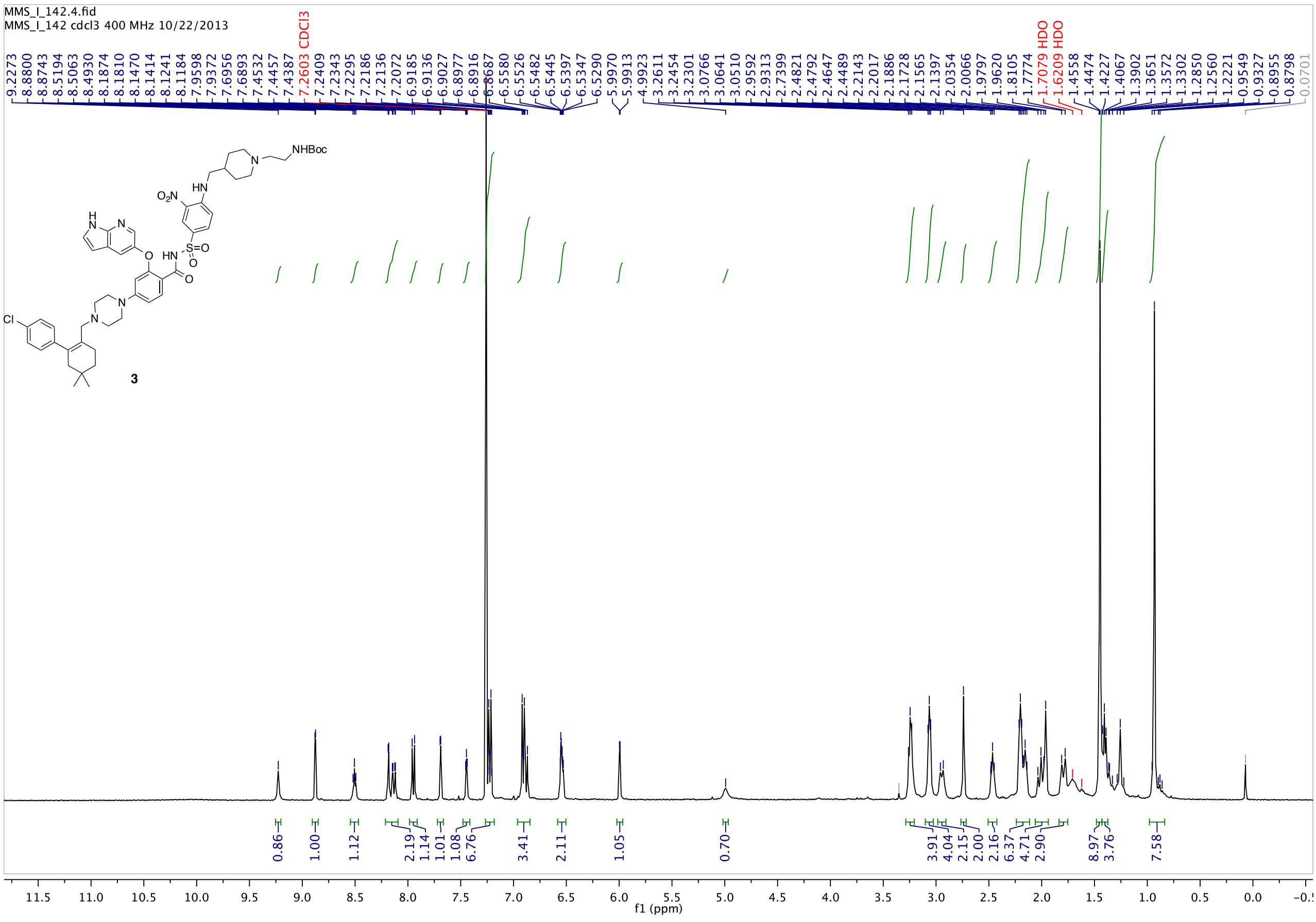


Figure S25. ^{13}C NMR Spectrum of **3**, recorded at 100 MHz in $CDCl_3$.

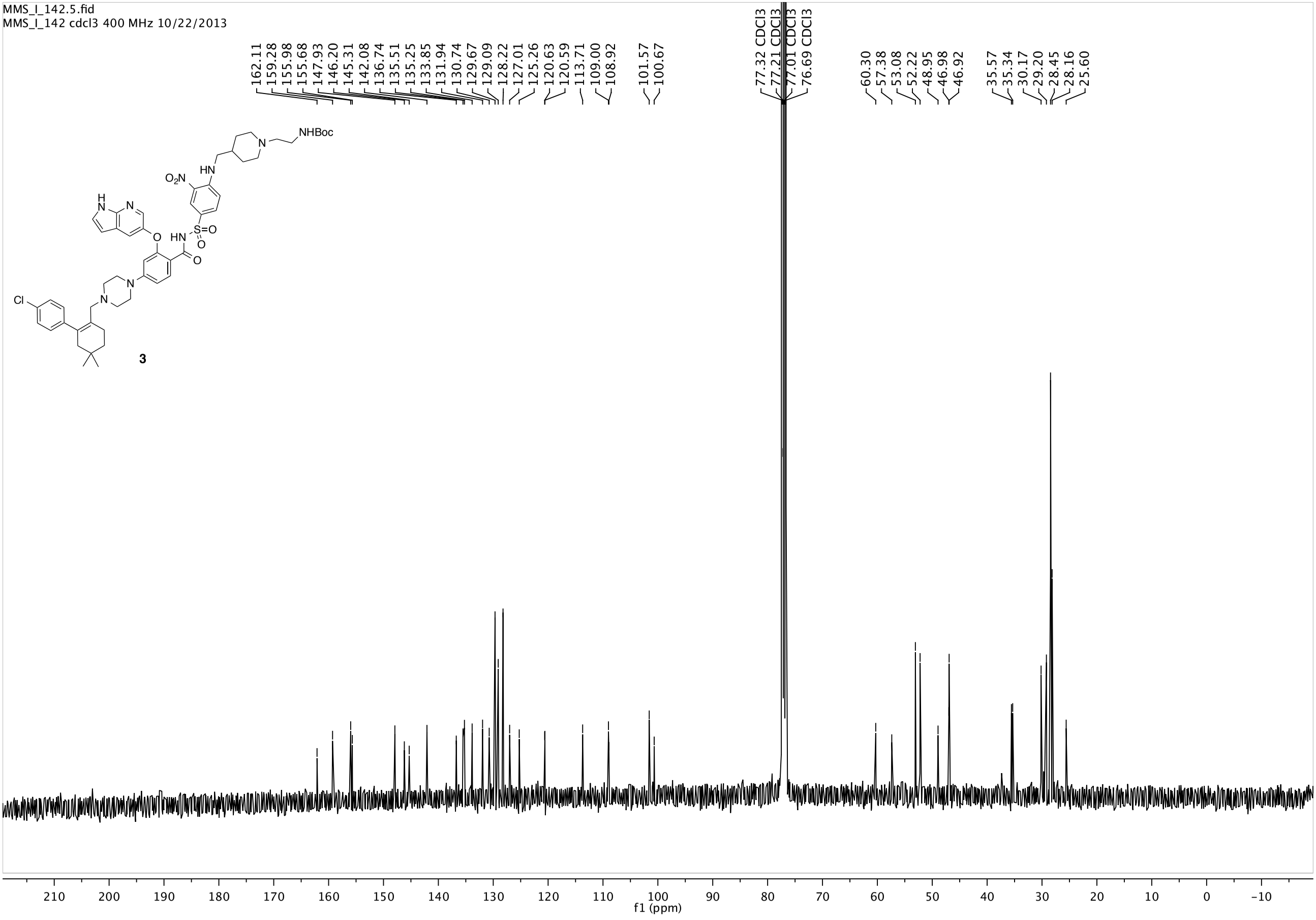
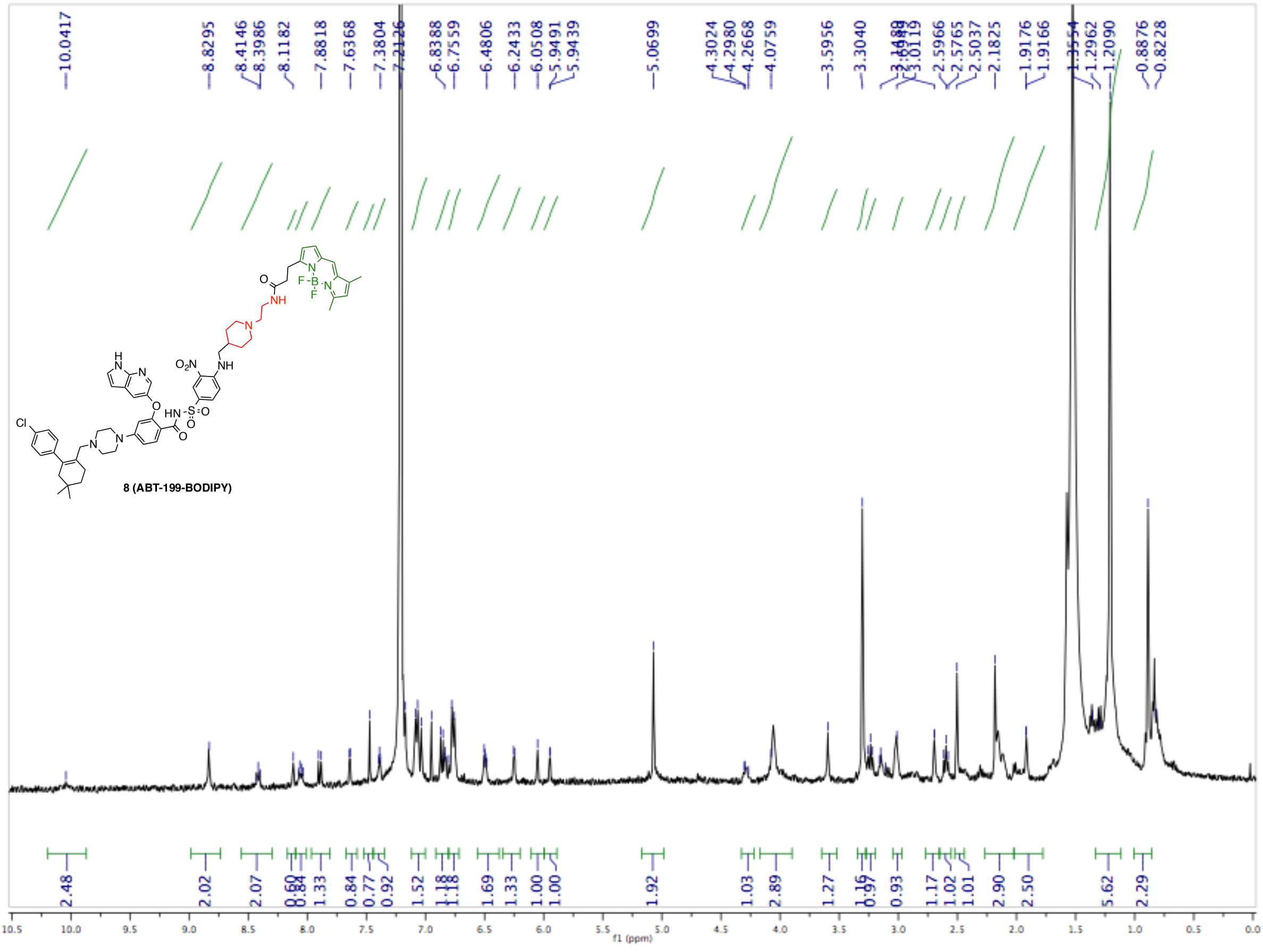


Figure S26. ^{1}H NMR Spectrum of **8 (ABT-199-BODIPY**, recorded at 400 MHz in $CDCl_3$.



8 (ABT-199-BODIPY)

Figure S27. ^{1}H NMR Spectrum of **7**, recorded at 400 MHz in DMSO-d_6.

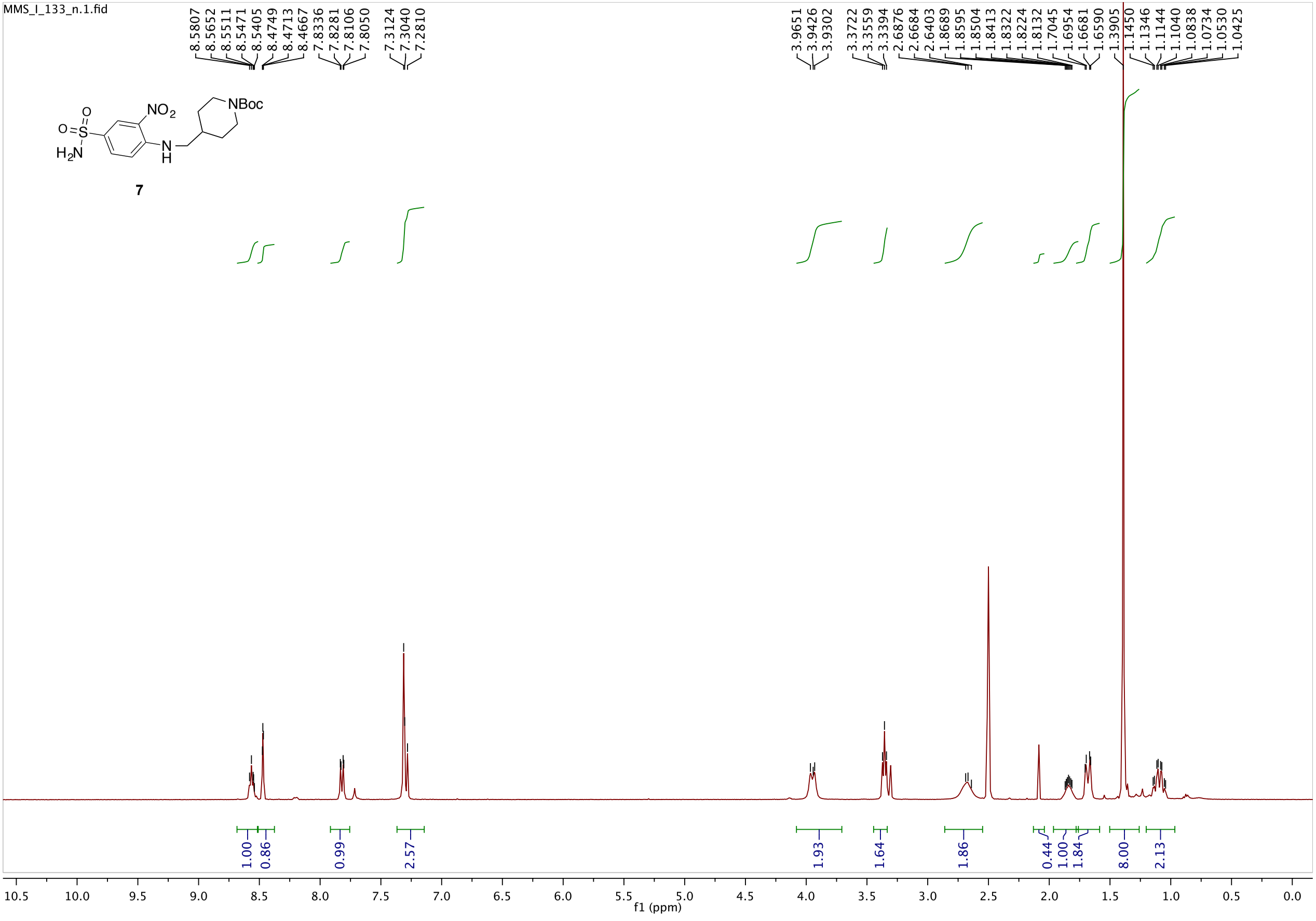


Figure S28. ^{1}H NMR Spectrum of **2**, recorded at 400 MHz in DMSO-d_6.